THE PROSPECTS FOR HUMAN GERMLINE GENETIC MODIFICATION: SCIENTIFIC AND BIOETHICAL CONSIDERATIONS

KEVIN RICHARD SMITH BSc (Hons), PGCE (Distinction)

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Signed .... [redacted] ..... Date 26/5/05

(Director of Studies)

ABSTRACT

This thesis considers the prospects for human germline genetic modification (HGGM) via a synthesis of scientific and bioethical concepts. Eleven published scientific and philosophical papers form the basis for the thesis. The published work deals with the science, technology and wider implications of gene transfer. A key theme underpinning the thesis is the notion that gene transfer is the central feature of two otherwise disparate bioscience fields – animal transgenesis and human gene therapy. Gene transfer is the route to transgenic animals, and human gene therapy depends upon gene transfer. The thesis argues that HGGM is now scientifically possible: the tools of animal genetic modification – pronuclear microinjection, sperm-mediated gene transfer (SMGT), nuclear transfer, etc – could in principle be applied to humans. However, serious technical obstacles remain to be overcome before HGGM could be considered a practical therapeutic proposition. Nevertheless, it is possible that gene transfer technologies will improve to the point at which it becomes easier and safer to perform HGGM than to carry out embryo pre-screening. In this futuristic scenario of expanded genetic knowledge coupled with effective gene transfer technology, HGGM would become the preferred route. This prospect of genetically modifying humans raises several vexing bioethical issues, including questions of responsibility towards future generations, difficulties of distinction between gene therapy and genetic enhancement, and the spectre of eugenics. Of the many philosophical approaches available to deal with such issues, utilitarianism is an internally consistent and highly consensible ethical system, and it is an approach that is often employed (explicitly or implicitly) in medical decision-making. This thesis argues for and adopts utilitarianism as a valuable, albeit imperfect, philosophical approach with which to address such issues. The utilitarian argumentation in this thesis generates several conclusions in the context of HGGM, including that: [a] the development of effective SMGT technology would largely nullify ethical concerns based on cost and access; [b] the embryo manipulation, genetic sequence alteration and animal experimentation entailed by the development and application of HGGM are all ethically permissible; [c] the notion of risk to future generations, although real, is not unique to HGGM, is likely to be technologically manageable, and is thus of lower ethical salience than *prima facie* consideration may suggest; [d] HGGM for enhancement (rather than for therapy) would be justified where used as prophylaxis against disease or disadvantage; and [e] the use of HGGM for entirely non-medical enhancements is unlikely to have positive utility, and is thus ethically objectionable, even in the futuristic scenario of effective SMGT becoming available. The overall conclusion of this thesis is that it would be unethical to proscribe medically effective HGGM.

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List of Abbreviations

AAV	adeno-associated virus
Ad	adenovirus
ADA	adenosine deaminase
AI	artificial insemination
APRT	adenine phosphoribosyltransferase
CF	cystic fibrosis
CKM	creatine kinase M
CO	chimeric oligonucleotide
COL1A1	collagen type I
DIC	differentiation interference contrast
DMD	Duchenne muscular dystrophy
DSA	deliberate sequence alteration
ESC	embryonic stem cell
GDP	gross domestic product
GH	growth hormone
GIT	gastrointestinal tract
GM	genetic modification
HPRT	hypoxanthine phosphoribosyl transferase
HR	homologous recombination
ICM	inner cell mass
IF	inhibitory factor
IF-1	inhibitory factor 1
IVF	*in vitro* fertilisation
LCR	locus control region
LOS	large offspring syndrome
LTR	long direct terminal repeat
MAC	mammalian artificial chromosome
MAR	matrix attachment region
MHC	major histocompatibility complex
MSC	mesenchymal stem cell
MT	metallothionein
NT	nuclear transfer
RVV	retroviral vector
SCID	severe combined immunodeficiency disease
SCID-X	severe combined immunodeficiency disease, X-linked
SMGT	sperm mediated gene transfer
TVC	total view consequentialism
YAC	yeast artificial chromosome

Chapter 1: Introduction

1.1 Background

The purpose of this thesis is to explicitly integrate and unify key aspects from the accompanying portfolio of published work (Section 1.2). The published work deals with the science, technology and wider implications of gene transfer applied to higher animals, including humans. This thesis is based on the concept that gene transfer is the central feature of two otherwise disparate bioscience fields – animal transgenesis and human gene therapy. In this (applied) context, gene transfer represents a technology for the genetic modification (GM) of higher animal genomes. Human germline genetic modification represents the ultimate potential use of GM technology. Human germline genetic modification forms the central focus of this thesis. Accordingly, this thesis considers the prospects for human germline gene therapy via a synthesis of scientific and bioethical concepts.

1.2 Published Work

1. Smith K.R. Sperm Cell Mediated Transgenesis: A Review. *Animal Biotechnology* 1999; **10**(1-2)**:** 1-13.
2. Smith K.R. Theoretical Mechanisms in Targeted and Random Integration of Transgene DNA. *Reproduction Nutrition Development* 2001; **41**(6) **:**465-485.
3. Smith K.R. Animal Genetic modification - A Utilitarian Response. *Bioethics* 2002; **16**(1)**:** 55-71.
4. Smith K.R. Gene Transfer in Higher Animals: Theoretical Considerations and Key Concepts. *Journal of Biotechnology* 2002; **99**(1)**:**1-22.
5. Smith K.R. The Role of Sperm-Mediated Gene Transfer in Genome Mutation and Evolution. *Medical Hypotheses* 2002; **59**(4)**:** 433-437.
6. Smith K.R. Gene Therapy: Theoretical and Bioethical Concepts. *Archives of Medical Research* 2003; **34**(4): 247-268.

7. Smith K.R. Gene Therapy: The Potential Applicability of Gene Transfer technology to the Human Germline. *International Journal of Medical Science* 2004; **1**(2): 76-91.

8. Smith K.R. & Spadafora, C. Sperm-mediated Gene Transfer: Applications and Implications. *BioEssays* 2005; **27**(5): 551-562.

Additionally, one further manuscript of relevance has been accepted for publication:

9. Smith K.R. Human Germline Genetic Modification - a Utilitarian Bioethical Perspective. This is a chapter in an edited book entitled *Trends in Gene Therapy Research,* published by Nova Science Publishers, New York. ISBN 1-59454-306-2. Publication is expected mid-2005.

1.3 Contribution to 'Original Knowledge' in the Published Work

The above publications review and analyse relevant areas of science and associated bioethics, and contain novel theoretical insights. Thus, each of the publications contributes to 'original knowledge'. The following sections (1.3.1-1.3.9) provide an outline of the contribution to original knowledge made by each publication, additional notes on citations and journal rankings, and a facsimile of each publication.

The published papers cited on pages 9-10 have been removed from the e-thesis due to copyright restrictions

1.3.1 Sperm Cell Mediated Transgenesis: A Review. *Animal Biotechnology* (1999)

This paper provides an original review of the status of a particular form of germline GM, sperm cell mediated transgenesis, or sperm-mediated gene transfer (SMGT). Contribution to original knowledge is made by evaluating and synthesising a range of key experimental and theoretical considerations. Thus, the paper functions as a meta-analysis of available information. Conclusions drawn include that: (a) 'augmented' SMGT, employing delivery systems such as liposomes or electroporation, is, conceptually, the more promising approach; (b) thorough evidence of the effectiveness of SMGT is required, by demonstration of transgene transmission to descendant animals; and (c) the potential importance of SMGT gives reason to pursue this GM approach, despite its theoretical and empirical limitations.

Notes: This paper has been cited several times by independent authors. The journal *Animal Biotechnology* is ranked 64th (from 131 journals) by impact factor in the 'Biotechnology' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.2 Theoretical Mechanisms in Targeted and Random Integration of Transgene DNA. *Reproduction Nutrition Development* (2001)

This paper provides an original review of the underlying mechanisms involved in the integration of transgenes. The paper's central theme is that gene targeting is the key to surmounting problems associated with randomly integrated transgenes. The potential for gene targeting in the GM contexts of animal transgenesis and gene therapy are explored. The paper takes a meta-analysis approach to available research findings and concepts. The paper postulates and adapts theoretical models of transgene integration, thus providing original contributions to knowledge. Analysis of the evidence supports the notion that multiple pathways of homologous recombination (HR) are operative in targeted transgene integration. Chimeric oligonucleotides (COs) are small, highly specialised transgene molecules that have recently been used for gene targeting. However, the mechanism of CO integration has not been elucidated. The paper proposes that CO integration is most likely to involve a form of HR, and goes on to postulate a novel putative model for this process. A simple novel model for nonconservative HR between circularly permuted transgene molecules is also presented. The paper suggests that gene targeting in non-selective systems would be a critically important development for future gene therapy applications. The overall conclusion is that further biomolecular investigation and concomitant model-building will be required in order to provide a basis for the control of HR such that gene targeting can be used routinely in GM applications such as gene therapy.

Notes: This paper has been cited by independent authors. The journal *Reproduction Nutrition Development* is ranked 20th (from 23 journals) by impact factor in the 'Reproductive Biology' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.3 Animal Genetic Modification - A Utilitarian Response. *Bioethics* (2002)

By considering the underlying scientific aspects, this paper explores the bioethics of animal GM. A utilitarian stance is adopted throughout. Although there exists much published literature dealing with ethical aspects of various forms of GM, a dedicated utilitarian response to animal GM based on a consideration of the underlying scientific aspects has not previously been published. Accordingly, this paper represents a primary contribution to original knowledge. After examining a number of ethical issues associated with animal GM, the paper comes to the radical conclusion that, while it would be wrong to prohibit animal GM per se, utilitarians ought to support a 'default prohibition' on transgenic experiments that entail significant suffering.

Notes: This paper has been cited elsewhere. The journal *Bioethics* is ranked among the top 10 journals by impact factor in the 'Ethics' category of the most recently available (2002) ISI Journal Citation Reports, as the number 6th top journal (from 28 journals).

1.3.4 Gene Transfer in Higher Animals: Theoretical Considerations and Key Concepts. *Journal of Biotechnology* (2002)

This paper integrates a broad range of scientific concepts to provide a unified account of gene transfer technology. The paper stresses that gene transfer technology comprises a common set of tools that can be used to achieve the disparate goals of animal transgenesis, somatic gene therapy and (in principle) germline gene therapy. Thus, the paper examines the different cell types - eggs, gametes, embryonic stem cells (ESCs), somatic cells - into which transgenes may be transfected *en route* to particular GM goals. Thus, this paper makes its main contribution to original knowledge by evaluating, synthesising and integrating key information and concepts. The paper draws attention to key aspects for attention, including improved control of target cell range, improved transgene uptake efficiencies, the ability to localise transgenes to the nucleus and improvements in gene targeting to enable the efficient integration of transgenes into chosen genomic loci. The overall conclusion made by the paper is that improvements in gene transfer are urgently required, particularly if hopes of effective gene therapies are to be realised.

Notes: This paper has been cited several times by independent authors. The *Journal of Biotechnology* is ranked 33rd (from 131 journals) by impact factor in the 'Biotechnology' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.5 The Role of Sperm-Mediated Gene Transfer in Genome Mutation and Evolution. *Medical Hypotheses* (2002)

This is an entirely original theoretical biology paper. Accordingly, it provides a significant contribution to original knowledge. The paper's central theme is that, if the reports of successful SMGT-based animal GM are not erroneous, it follows that the genome of sexually reproducing animals, including humans, should be subject to alteration by naturally occurring exogenous DNA sequences carried by sperm cells. The paper considers experimental findings from GM experiments combined with key theoretical concepts surrounding transgene integration. The overall conclusion made is that geneticists would be wise to be vigilant to the potential evolutionary and medical consequences of SMGT.

Note: The journal *Medical Hypotheses* is ranked 60th (from 74 journals) by impact factor in the 'Medicine, Research and Experimental' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.6 Gene Therapy: Theoretical and Bioethical Concepts. *Archives of Medical Research* (2003)

Human gene therapy, particularly germline gene therapy, represents an ultimate use of GM technology. This paper provides a meta-analysis of key research findings and theoretical concepts in the context of gene therapy, and these scientific considerations are integrated with a bioethical analysis of gene therapy, present and future. The bioethical approach is explicitly utilitarian. Although there exists much published literature dealing with ethical aspects of gene therapy (especially somatic gene therapy), a dedicated utilitarian response to all forms of gene therapy based on a unified consideration of the underlying scientific aspects has not previously been published. Accordingly, this paper provides an original contribution to knowledge. A novel aspect of this paper is that it considers how a common set of gene transfer tools can in principle be used for both somatic and germline forms of gene therapy. Scientific aspects of germline gene therapy have not hitherto been widely considered in peer-reviewed literature. In this respect, the paper concludes that germline GM could be achieved by relatively minor adaptations of presently available animal GM technology. However, from a bioethical perspective, the paper suggests that human germline gene therapy should not be undertaken at the present time, in view of the relatively low efficiency and safety of current gene transfer technology. However, the paper makes the radical claim that germline gene therapy, together with some forms of genetic enhancement, promises great benefits to humanity and thus ought to be pursued.

Notes: According to information supplied (in July 2004) to the author by the *Archives of Medical Research* journal, this paper appeared among the 25 most highly requested articles in the journal (of all time), as the number 23rd top article in the journal. The journal itself is ranked 62nd (from 74 journals) by impact factor in the 'Medicine, Research and Experimental' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.7 Gene Therapy: The Potential Applicability of Gene Transfer Technology to the Human Germline. *International Journal of Medical Science* (2004)

This paper explores the theoretical possibility of applying gene transfer methodologies to the human germline. Transgenic methods of genetic manipulation are reviewed and evaluated in terms of the possibilities for applying such methods to the human germline. Thus, this paper represents a uniquely focused analysis and synthesis of contemporary science and technology. The conclusion is drawn that human germline gene therapy remains, for all practical purposes, a future possibility that must await significant and important advances in gene transfer technology. Bioethical considerations are deliberately set aside in this paper, to permit a purely scientific account to be developed. By serving as a unique analysis, this paper provides a significant contribution to original knowledge.

Note: The *International Journal of Medical Science* was not established at the time when data was being collected for the most recently available (2002) ISI Journal Citation Reports.

1.3.8 Sperm-mediated Gene Transfer: Applications and Implications. *BioEssays* (2005)

This paper was co-authored by Dr Corrado Spadafora of the Italian National Institute of Health. Dr Spadafora is a pioneer in the field of SMGT, and has published a large body of empirical and theoretical work on SMGT. Dr Spadafora and I contributed equally to the manuscript. The paper provides a substantial review and analysis of SMGT. Recent advancements in the technology are highlighted, including the innovation of transgenICSI and the development of *in vivo* gene transfer. Drawing upon various research findings, a possible mechanism of SMGT-specific transgene integration is posited. A unique feature of this model is the notion that transgenic animals may be generated via exogenous RNA rather than DNA. The establishment of a plausible mechanism of SMGT-specific transgene integration is significant, because clarification of the molecular basis of SMGT processes was one of the main requests that the scientific community made years ago when the SMGT controversy first arose. In addition to detailed molecular-level consideration of transgene behaviour, the paper considers a variety of possible implications of SMGT, including those that would arise if this process should occur spontaneously. All these aspects have never been deeply evaluated before in the frame of one broad review/analysis article. Thus, this paper represents a significant contribution to original knowledge.

Note: the journal *BioEssays* is ranked 1st (from 62 journals) by impact factor in the 'Biology (General)' category of the most recently available (2002) ISI Journal Citation Reports.

1.3.9 Human Germline Genetic Modification - a Utilitarian Bioethical Perspective. *Trends in Gene Therapy Research* (2005 – in press.)

This paper and Chapter 7 of this thesis are closely related. The paper is premised on the view that it is inevitable that both technology and genetic knowledge will advance to the point at which potential human germline genetic modification applications become real possibilities. Key ethical aspects of human germline genetic modification are identified and explored, including safety, consent, public acceptability, cost and access, human embryos, deliberate sequence alteration, and genetic enhancement. The paper concludes that it would be unethical to proscribe medically effective and safe human germline genetic modification. The paper contributes to original knowledge by blending a utilitarian approach with key scientific concepts to produce original conclusions.

1.4 Thesis Outline

In addition to appraising the published work (above), Chapter 1 provides an introductory account of gene transfer from a scientific and a bioethical perspective. The organising theme of gene transfer technology representing a common set of tools is outlined. The bioethical approach used in this thesis, utilitarianism, is briefly introduced.

Chapter 2 reviews the scientific basis of gene transfer (transfection). The basis for the genetic modification of higher animal genomes lies in the range of transfection approaches that can be used to deliver exogenous DNA to host cells. A broad range of transfection approaches exists, and this armamentarium may be applied to a broad range of host cell types, from germline cells to embryonic cells to terminally differentiated cells. Thus, gene transfer is the route to transgenic animals, and human gene therapy depends upon gene transfer.

A key distinction between the outcomes of gene transfer lies in the nature of transgene uptake: in most cases where transgene sequences integrate into the host DNA, the integration loci are essentially random. However, gene targeting is also possible, whereby transgenes are induced to integrate into precisely defined genomic loci. Clearly, gene targeting, where possible, is to be preferred over random integration, given the inherent degree of control available from the former process. Gene targeting is examined in Chapter 3.

Chapter 4 considers the scientific and technical feasibility of applying GM techniques to the human germline. Germline gene therapy is now scientifically possible: the tools of animal GM (pronuclear microinjection, sperm-mediated gene transfer, nuclear transfer, etc) could in principle be applied to humans. However, serious technical obstacles remain to be overcome before germline gene therapy could be considered a practical proposition.

Chapter 5 considers answers to a crucial question concerning germline gene therapy: Why do it? Given the low transfer efficiencies and safety risks available at present

(i.e. extrapolating from animal transgenesis), candidate disorders would have to be severe and otherwise unavoidable. However, there is in effect a 'golden rule' applying to disorders potentially amenable to germline gene therapy: in any disorder with enough molecular knowledge available to allow the prospect of germline gene therapy, that same knowledge will also be sufficient to allow detection of the disease-causing sequences via embryo pre-screening. It is possible that gene transfer technologies will improve to the point at which it becomes easier and safer to perform germline gene therapy than to carry out embryo pre-screening. In this futuristic scenario of expanded genetic knowledge coupled with effective GM technology, germline GM would become the preferred route.

Chapter 6 introduces bioethical concepts. Non-utilitarian forms of ethical reasoning are examined. Utilitarianism is presented as a plausible ethical approach to modern biomedical issues. The use of such a system is explored, as are its key problems. The resultant chapter is an original general account of utilitarianism in the context of biomedical issues.

Chapter 7 discusses bioethical issues specifically arising from the possibility of human germline genetic modification. In both its deliberate and accidental guises, human germline genetic modification raises several vexing bioethical issues, including questions of responsibility towards future generations, difficulties of distinction between gene therapy and genetic enhancement, and the spectre of eugenics. Thus, human germline genetic modification is far more contentious than somatic gene therapy.

Chapter 8 concludes the thesis. It appears inevitable that both technology and genetic knowledge will advance to the point at which potential human germline genetic modification applications become real possibilities. I argue that it would be unethical to proscribe medically effective and safe human germline genetic modification. Biomedical scientists and bioethicists will undoubtedly bear a heavy burden of responsibility for objectively informing society of the probable consequences of using or misusing tomorrow's tools of human germline genetic modification.

1.5 The Science of Gene Transfer

During the 1970s it became possible to introduce exogenous DNA constructs into higher eukaryotic cells *in vitro.* Mammalian (germline) transgenesis was first achieved in the early Eighties, mice being the subject species. Transgenic members of a wide range of animal (and plant) classes and species have now been produced, including amphibians, cattle, chickens, fish, insects, nematodes, pigs, rabbits, and sea urchins. Transgenic animals may in principle be utilised in either of two broad ways: (a) as models for fundamental or applied scientific study; and (b) as novel sources of pharmaceutical agents, or human-compatible organs for xenotransplantation.

Gene transfer methods have been used in somatic gene therapy attempts on humans since 1990. Gene therapy approaches have so far focused primarily on monogenic disorders and cancers. To date, limited clinical success has been achieved. However, somatic gene therapy is in its infancy and holds great promise for the future.

Gene transfer in higher eukaryotes may in principle be applied directly for therapeutic purpose to the human germline. In contrast to somatic gene therapy, human germline gene therapy has never (yet) been attempted with humans (unless one includes the transfer of foreign mitochondria during some forms of artificial fertilisation). human germline gene therapy may be viewed as the most advanced potential use of gene transfer technology possible. Gene therapy represents the ultimate form of genetic modification, and it has the potential to eventually revolutionise the treatment and prevention of human disease. However, the prospect of human germline gene therapy raises serious, novel and perplexing ethical concerns.

This thesis considers: (a) key technical and scientific aspects of gene transfer; (b) human germline gene therapy possibilities; and (c) major bioethical issues associated with the possibility of human germline gene therapy.

1.6 Bioethics and Gene Transfer

It is commonly held that one's ethics are a matter of personal belief, and that there are as many ethical positions as there are individuals. In bioethics this view has some weight: it enjoins us to respect the views of patients themselves, and it supports the democratisation and plurality of membership of ethics committees. Nevertheless, the view that ethics are purely a matter of individual belief is highly problematic, primarily because it provides absolutely no guide for deciding what course of action to take when faced with ethical dilemmas. (As a member of an ethics committee, one may ponder: on what basis ought I to proceed?) One possible solution to this problem is an appeal to intuition: we ought to let our intuitive responses guide our ethical judgements. However, while this approach may have its uses in everyday life, it is severely limited in the context of bioethics. Scientific and technological advances produce novel, highly esoteric ethical problems. It seems clear that our intuitive ethical responses, insofar as such responses are an inherent part of our evolved human nature, simply cannot cope reliably with novel issues such as human genetic modification, human stem cell research, human sex selection, human cloning, or other similar possibilities thrown up by contemporary science and technology.

As an alternative to intuition, various metaphysical and religious doctrines hold ethical views on biomedical issues. In particular, the debate about human genetic modification abounds with rhetorical pleas, such as those concerning the claimed reprehensibility of "playing God." While such views have rhetorical force, they are rendered ineffective as general guides to ethical action by (a) the major problem of fundamental lack of agreement between different religions, and (b) their lack of moral purchase on secular persons. Thus, as far as bioethics are concerned, there may be little hope of taking the discussion any further with those who hold such views.

I suggest that, if progress is to be made, judgements of ethical acceptability/reprehensibility need to focus on the predictable *consequences* arising from a course of action, where 'good' consequences are those in which wellbeing – in the form of happiness – is maximised. This approach, often referred to as 'utilitarianism', is an internally consistent and highly consensible ethical system, and

it is an approach that is often employed (explicitly or implicitly) in medical decision-making.

In this thesis I adopt a utilitarian perspective when considering the ethics of human germline gene therapy. I hold utilitarianism to be a powerful ethical approach in this context. However, my purpose is not one of advocacy: this thesis does not claim to establish utilitarianism as being the only valid – or even necessarily the best – ethical approach to human germline gene therapy. Instead, I employ utilitarianism as a *tool* for the exploration and evaluation of key bioethical issues arising from the future prospect of human germline gene therapy. Doubtless, the scientific issues surrounding human germline gene therapy would be addressed in a different manner using an alternative moral system or approach. For example, a Catholic theologian would no doubt use entirely different arguments and come to quite different conclusions from those presented herein. However, the theological approach would form the basis for another thesis on human germline gene therapy, not the present one. Of course, it would be legitimate (and interesting) to compare the arguments and conclusions that alternative ethical approaches might generate in the context of human germline gene therapy. However, a single thesis would be insufficient to accommodate such an endeavour.

Thus, although the conclusions reached in this thesis are designed to be consistent with utilitarianism, those who reject the underlying principles of utilitarianism may legitimately reject these conclusions.

Chapter 2: Molecular and Cellular Aspects of Genetic Modification

2.1 Introduction

Germline GM requires that transgenes be delivered while the host organism is at a very early stage of development. Developmental stages applicable for transgene delivery in principle include gametes (oocytes or sperm) and early embryos (zygotes or early morulae). As an alternative to these natural biological entities, gene transfer may be performed on cells maintained *in vitro* that are able to genetically contribute toward an embryo. Such cells include (a) embryonic stem cells (ESCs) and (b) somatic cells the nuclei of which are placed into recipient zygote cells by nuclear transfer (NT).

2.1.1 Microinjection

Jon Gordon in 1980 demonstrated that exogenous DNA could be introduced into the germline simply by the physical injection of a solution of cloned DNA into zygote pronuclei (Gordon et al. 1980). Subsequently, pronuclear microinjection has become the most widely used method of germline gene transfer, despite the fact that it remains an intrinsically costly and laborious approach. The technique is most established with mice, however gene transfer via pronuclear microinjection has also been carried out with a wide range of other mammals including rats, rabbits, and agricultural animals. Accordingly, it is to be expected that the human zygote should in principle be similarly amenable to gene transfer via pronuclear microinjection.

The microinjection technique is intrinsically simple, although it requires expensive equipment and high levels of skill (Hogan et al. 1994). A fine glass needle is loaded with DNA solution. Under the microscope, the needle is guided through the cytoplasm towards one of the zygote's pronuclei. A nanolitre quantity of DNA solution is injected, bringing typically two hundred DNA molecules into the pronucleus. Some zygotes die following microinjection, probably due to the physical trauma of being penetrated by the micropipette, however the majority of zygotes

survive. Surviving zygotes are transferred to the uterus of a surrogate mother. For mice, typically around 15% (range 0-40%) of the offspring will be transgenic. These values should be interpreted with caution however, because microinjected embryos are prone to: (a) lysis immediately following microinjection; (b) death *in vitro* following microinjection; and (c) death *in vivo* during gestation in the recipient female. Accordingly, when the numbers of zygotes used is taken into account, the efficiency values are less impressive: typically only ca. 2% (range 0-6%) of microinjected zygotes will become transgenic animals (Bagis and Papuccuoglu 1997; Brinster et al. 1985; Page et al. 1995). Moreover, reported efficiency values are significantly lower for most mammals other than mice. Compared with murine efficiencies, the rate of porcine and ovine transgenesis through pronuclear microinjection is ca. 5-fold lower, and bovine efficiencies are ca. 17-fold lower (Hirabayashi et al. 2001; Seamark 1994; Wall 2002; Wall et al. 1997).

Beyond the use of microinjection for transgene delivery to zygotes, it is worth noting that microinjection is also effective as a means to deliver transgenes to the genomes of non-zygotic cells, such as ESCs or somatic cells for NT. However, given that the normal use of such cells is with *in vitro* selection systems requiring *en masse* transgene delivery to millions of cells, non-zygote microinjection is not a practical means of producing transgenic animals.

Oocytes may in principle be suitable targets for transgenesis. However, fundamental practical problems have so far precluded their use. Oocytes collected following ovulation would have to be fertilised after transgenic manipulation. This would entail the use of *in vitro* fertilisation (IVF). Potentially-transgenic oocytes would thus have to endure a further, extensive *ex vivo* procedure. Since this could only be detrimental to the oocytes, it is difficult to see any role for transgenesis directed at this level rather than at the zygote. Attempts to produce transgenic animals by DNA microinjection into oocytes have never or very rarely resulted in success (Houdebine 2002a).

Transgene DNA delivered by microinjection integrates into the host cell's endogenous DNA. Integration is random and usually occurs at only one chromosomal site in each transgenic. The number of copies of the transgene at an integration site may range from one to thousands. For multiple copy inserts, the most common

arrangement is an array of molecules joined head-to-tail (Smith 2001). Less usually, tail-to-tail and head-to-head arrangements occur. Deletions, duplications and other rearrangements may occur at the junctions between chromosomal and transgenic DNA sequences (Bishop 1996). Only a limited amount is known about the mechanisms of transgene integration.

Transgene sequences integrating randomly into the host genome tend to give poor levels of expression, or exhibit inappropriate expression, in the form of temporally or spacially (ectopic) aberrant expression. The primary reason for such problems is the 'position effect', whereby the particular genetic environment at any point of insertion is likely to influence the expression of the integrated transgene. In some cases the remedy lies with transgene design: for example by ensuring that an appropriate enhancer sequence is included in the transgene construct. Beyond this, it may be possible to insulate a gene from the position effect. Matrix attachment regions (MARs) are sequences which, when placed on either side of a gene within a transgene construct, appear to allow the gene(s) within an integrated transgene to occupy a separate chromosomal domain. Alternative 'insulator' sequences (for example, locus control regions (LCRs)) are also under investigation. Ultimately, however, the best solution to transgene expression problems would be to avoid the position effect entirely. This is achievable through gene targeting: a transgene targeted to a chosen genomic locus will by definition avoid the position effect. Reliable ways of germline gene targeting do exist. However, gene targeting is not possible at present with pronuclear microinjection, due to the inability to select for zygotes that contain rare targeted integrations.

There is no particular restriction on the size of DNA molecule used for microinjection. Yeast artificial chromosome (YAC) based transgene constructs consisting of >100 kb of DNA have been successfully introduced into the mouse germline by pronuclear microinjection (Lamb and Gearhart 1995). Because there are no special problems with microinjecting large transgene constructs, it is possible to incorporate structural gene-plus-promoter (plus other potentially useful sequences such as enhancers) combinations into the host genome. Indeed, it may become possible (pending development of the necessary transgene constructs) to microinject autonomous artificial 'mini-chromosomes', (mammalian artificial chromosomes,

MACs) complete with centromeres, telomeres and replication origins in addition to structural genes, promoters and enhancers. These specialized constructs would be expected to give a number of benefits compared with integrated transgenes, the most important of which would probably be the absence of chromosomal position-effects on transgenic expression (Sgaramella and Eridani 1996).

It has recently become apparent that the majority (ca. 85%) of pronuclear microinjection-derived transgenic founders are mosaics of transgenic and nontransgenic cells (Whitelaw et al. 1993). This surprising finding may be explained by postulating that (endogenous) DNA replication is required for chromosomal integration. The relatively few nonmosaic founders that do arise may plausibly be accounted for by assuming that the nontransgenic daughter cell (in a 2-cell zygote) has died. This explanation is consistent with the fact that microinjected DNA *per se* has a negative effect on embryo viability (Brinster et al. 1985). In ca.15%-25% of mosaic founders, the foreign DNA apparently integrates at later stages of the embryo cell replication, resulting in mice that contain the transgene in varyingly small proportions of their cells.

As with other mosaics, transmission of the transgene is dependent upon the existence and extent of germline colonisation by transgene-containing cells. In the vast majority of cases where transmission occurs (whether from fully transgenic founders, from mosaic founders or from subsequent generations of transgenics), the transgene is inherited in a stable Mendelian fashion, although exceptions have been found (Palmiter et al. 1984). Due to the hemizygous nature of transgene insertion, even a nonmosaic founder will transmit its transgene to only (on average) 50% of its offspring.

Given that pronuclear microinjection seems to be effective in all mammalian species, it seems certain that the technique could in principle be readily applied to human germline gene therapy.

2.1.2 Retroviral Transfer

Retroviruses are found in many species, including most mammals (Lazo and Tsichlis 1990). Retroviruses have RNA as their genetic material. Following infection, the virus-encoded enzyme, reverse transcriptase, transcribes the RNA. The resultant single-stranded DNA (ssDNA) is replicated as double-stranded DNA (dsDNA). The dsDNA viral genome has the important property of being able to linearly integrate (as a provirus) into the chromosomal DNA of the host cell. The site of integration appears to be essentially random, although there may exist some preferred integration sites, at least in some cell types (Laufs et al. 2003).

The genome of retroviruses can be manipulated to carry exogenous DNA. Zygotes may be incubated in media containing high concentrations of the resultant retroviral vector. Alternatively, retroviral vector-producing cell monolayers may be used, upon which zygotes are co-cultivated. In either case, up to ca.90% of (surviving) embryos will be infected. Following zygote transfer into pseudopregnant females, the infected embryos should give rise to transgenic offspring. Molecular genetic analysis of transgenics produced in this way usually show integration of a single proviral copy into a given chromosomal site. Rearrangements of the host genome are normally restricted to short direct repeats at the site of integration. In many embryos the germline cells contain viral integrants: thus, transmission of the transgene to the next generation will often occur. Methods have also been developed to allow infection into postimplantation embryos. In this context, virus uptake is effective for many somatic cell lines, however germline cells are infected at low frequency, due to a high level of mosaicism (Braas et al. 1996; Morgan and French Anderson 1993).

The major advantages of retroviral vectors (RVVs) are: (a) the efficiency of gene transfer; (b) the low equipment/expertise requirements; and (c) the unitary form of integration.

However, retroviral vectors are limited or problematic in a number of respects. Possibly the most fundamental limitation is the inability to carry large transgenes. The

upward length of transgene sequence that may be incorporated into the retroviral genome is in the order of 9-10 kb (Hu and Pathak 2000).

The degree of control over the expression of transgenes carried by RVVs is another problem. There is less scope for control in RVV-based transgenes compared with the control possible in transgenes delivered by physical methods such as pronuclear microinjection. Since RVVs integrate into the host DNA in a largely random fashion, each particular chromosomal integration site is likely to have a particular effect on the transcription of the transgene. Additional factors also interfere with expression, such as promoter efficiency in particular tissues, RNA message efficiency, and DNA methylation. The net result is inconsistency of expression (Pannell and Ellis 2001; Pannell et al. 2000; Swindle and Klug 2002). Unfortunately, because transgene expression in the provirus is driven by the viral 5' long direct terminal repeat (LTR), it is problematic to engineer into the construct the ability for it to be controlled tissue-specifically, temporally, or by external (clinical) influence. Nevertheless, various tissue-specific and non-specific control elements have been incorporated into RVVs, and the efficiency of these promoters in particular cell types is the subject of intense investigation (Hoatlin et al. 1995; Hu and Pathak 2000; Pannell and Ellis 2001; Solly et al. 2003; Vile et al. 1996).

Given the essentially random (nonhomologous) mode of integration of natural retroviruses, retroviral vectors do not appear to hold much promise for applications in which gene targeting is required. However, using integration-deficient retroviral vectors, Ellis and Bernstein (Ellis and Bernstein 1989) were able to target genomic loci such that vector sequences homologously recombined with endogenous sequences. However, the frequency of targeting was very low (approximately 1 targeted event per $3x10^6$ infected cells).

Only actively dividing cells are infectable by most retroviral vectors (Miller et al. 1990). This means that, in practice, infection does not occur at high efficiency until around the eight-cell stage. The mosaicism arising in such embryos would be of a more profound variety than that associated with pronuclear microinjection, because each resultant individual would likely consist of several cell types, with each cell type

containing the transgene in a different genomic site. However, a new class of retroviral vectors based on lentiviruses has recently been developed. Lentiviruses are unusual amongst retroviruses in that they are able to infect quiescent cells. Transgenic mice have been produced at high efficiency (70-80% of animals born) by exposing zygotes to lentivirus-based vectors, thus avoiding the problems of mosaicism normally associated with retroviral vectors (Ikawa et al. 2003; Lois et al. 2002; Pfeifer et al. 2002).

It is possible for integrated retroviral DNA to be spontaneously reactivated (Weiss et al. 1985), leading to the production of new integration within the DNA of the cell, to new infection of other cells or potentially to infection of other individuals. Such instability again may result in transgene expression problems and safety concerns (Cornetta et al. 1991; Gunter et al. 1993; Temin 1990). However, retroviral vectors may be engineered such that they lack the genetic sequences required for a normal life cycle (Vile et al. 1996). The creation of such 'defective' retroviral vectors goes a long way towards curing instability-related expression and safety problems. However, the risk of reactivation can probably never be completely eliminated, because complementation by a competent 'helper' retrovirus cannot be ruled out. In a controlled laboratory environment this may well represent only a minor concern. However, for humans treated with gene therapy (somatic or germline) entering the outside environment, the risk may be more acute. In addition to the risks of releasing infectious agents into the general environment, there are concerns for patients who have been treated with retroviral vector-based gene therapeutic agents. In such cases, retroviral reactivation could conceivably lead to oncogenesis.

Despite the limitations and safety concerns referred to above, retrovirus-mediated gene therapy has already been used in a number of somatic gene therapy attempts, and appears to hold a good deal of promise in this regard. Additionally, retrovirus-mediated gene transfer has been used successfully for (nonhuman) germline modifications. However, concerns over the safety and effectiveness of retroviral vectors are likely to limit the use of the approach for human germline gene therapy.

2.1.3 Other Viruses

Viruses other than retroviruses hold some promise as germline vectors. Adenoviruses (Ad) have a number of properties that make them potential candidates for human germline gene therapy. Ad are able to infect dividing and quiescent cells. Ad are able to carry large transgenes (up to c. 38kb) without adversely affecting their infectivity (Bett et al. 1993). Ad have a low host cell/species-specificity, suggesting that various cells (ESCs, somatic cells for NT, zygotes) from a wide range of species may be amenable to Ad-mediated gene transfer. The production of transgenic mice following the infection of zona-free zygotes with a replication-defective Ad vector has been reported (Tsukui et al. 1996). This intriguing result suggests the possibility of a promising new strategy for germline GM. However, further research is required in order to determine basic parameters and maximise the efficiency of this form of adenoviral gene transfer (Gordon 2002).

Like the adenoviruses upon which they are naturally dependent, adeno-associated viruses (AAV) exhibit low host cell specificity and are able to infect both dividing and quiescent cells. A unique property of AAV is the ability to undergo site-specific genomic integration in chromosome 19, mediated via the viral Rep protein. This property offers predictable transgene expression. AAV gene delivery vectors are under intensive development, and it is to be hoped that some vectors might prove useable with germline cells (Carter and Samulski 2000; Lai et al. 2002a; Monahan and Samulski 2000).

Retroviruses, adenoviruses and adeno-associated viruses are certainly not the only types of viruses that are under scrutiny as transgene vectors. No single virus has the necessary characteristics for all applications. Not even a fraction of the possible types of viruses has been assessed for potential utility in germline transgenesis. As virology research continues, it is to be expected that other types of viruses will be added to the current store of potential germline gene transfer vectors.

2.1.4 Liposome-mediated Gene Transfer

Liposomes are artificial vesicles that can act as delivery agents for exogenous materials including transgenes (Ilies and Balaban 2001; Nicolau et al. 1987; Watwe and Bellare 1995). Like their natural cellular counterparts, liposomes comprise a lipid bilayer similar to that of natural cells, surrounding a small volume of aqueous solution. Liposomes for use as gene transfer vehicles are prepared by adding an appropriate mix of bilayer constituents to an aqueous solution of DNA molecules. A self-organising process creates discrete spheres of continuous lipid bilayer membrane enveloping a small quantity of DNA solution. (Felgner 1996; Mahato et al. 1997). When they come into contact with a target cell, liposomes interact with the cell membrane to allow the exogenous DNA to enter the cell, by endocytosis or membrane fusion (Connor et al. 1984; Lamb 1993).

It is difficult to generalise about liposomes, since they have been produced in a wide diversity of forms. Differences include: (a) size (nanometer to micrometer scale); (b) shape (spheres or lipoplexes); (c) charge (positive or negative); (d) coat structure (bilayer or multilamellar); and (e) the presence of particular accessory molecules (e.g. Sendai glycoprotein, cell-specific ligands, nuclear localisation signals).

The capacity of liposomes varies greatly depending upon type. However, the size (length) of transgene that may be carried is always much lower than with pronuclear microinjection.

Some liposome-based gene transfer systems are often able to deliver transgenes to the cytoplasm with a reasonable degree of efficiency. However, only in a minority of cells will transgenes reach the nucleus. Amongst transgene molecules that do reach the nucleus, only a small minority integrate into the host cell chromosomes. This relatively low integration frequency renders liposome-mediated transfection impractical for use with mammalian zygotes, although some experimental success has been claimed (Carballada et al. 2002).

Although liposomes are not suitable for the delivery of transgenes to zygotes, liposomes may be used to deliver transgenes to ESCs or somatic cells for NT, thus producing germline alterations (Pain et al. 1999). This approach has the advantage of being an *en masse* gene transfer tool, in that many millions of cells may be treated simultaneously. Moreover, given that the host cells are maintained *in vitro*, it is possible to use *in vitro* selection to enrich for cells containing rare desired integration outcomes. Accordingly, liposomes may be incorporated into germline gene targeting protocols.

Finally, an indirect approach to the germline via the zygote remains a putative possibility, where liposomes would be used to deliver transgenes to sperm cells. Some success has been claimed for the *in vitro* use of liposomes in this way (Smith 1999). Liposomes may also be able to deliver transgenes to sperm *in vivo* (Sato et al. 1999a). Sperm-mediated gene transfer is discussed in Section 2.1.7 (below).

2.1.5 Electroporation

Electroporation is a process by which high-intensity electric field pulses temporarily destabilise cellular membranes. During the destabilisation period, DNA molecules present in the surrounding media are able to permeate the cell's external and internal membranes to enter the cytoplasm and nucleoplasm (Lurquin 1997).

Electroporation provides a fast and inexpensive means of introducing exogenous DNA into cultured cells. The process can be equilibrated to yield copy numbers (of integrated transgenes) of between 1 and 20 copies per genome - an advantage compared with microinjection. Large transgene molecules ($\geq$ 150 kb) can be transferred. In addition to the advantages of being able to transfer large conventional transgenes, the DNA transfer capabilities of electroporation may in future allow transfer of MACs, as with pronuclear microinjection. The main drawbacks of electroporation are that: (a) specialised equipment is required; (b) each cell type and culture system requires fairly extensive empirical optimisation; and (c) typically only

around 0.01% of treated cells show genomic integration of transgene (Chang 1992; Lurquin 1997; Potter and Cooke 1992).

In terms of germline GM, electroporation is an effective method of introducing exogenous DNA into ESCs (Chu et al. 1987). The advantage of electroporation over microinjection in the context of ESCs is that electroporation allows the *en masse* treatment of large numbers of cells. This is extremely useful where a rare integration event requires selection from a background of unwanted integrations, as in gene targeting. Similarly, electroporation has been successful with nuclear transfer transgenesis (McCreath et al. 2000; Schnieke et al. 1997).

The relatively low efficiency of electroporation renders it impractical for use with mammalian zygotes. The best superovulation protocols deliver around thirty zygotes per animal for mice and pigs, and ten for cattle and sheep (Wall et al. 1992). Extrapolating from these figures, a 0.01% efficiency rate would necessitate on average 300+ mice or pigs, and ca. 1,000 cattle or sheep in order to obtain just one transgenic. However, for (mammalian) species that produce large numbers of easily recovered zygotes, electroporation shows more promise in terms of transgenesis. Many fish species are potentially useful in this respect, and some successes have been claimed. For example, Murakami et al. (Murakami et al. 1995) report the successful use of electroporation to create transgenic medaka, as do Ono et al. (Ono et al. 1997), with the latter also reporting successful transmission of transgenes to F_1 progeny.

An attractive putative use of electroporation for transgenesis would be to enhance the transfer of transgene molecules into sperm cells (see Section 2.1.7, below). For example, Gagne et al. (Gagne et al. 1991) report an increase from 12% to 19% of transgenic bovine blastocysts when electroporation is included in an otherwise passive sperm-DNA uptake protocol. Similar findings were reported by Rieth et al. (Rieth et al. 2000), again with transgenic bovine blastocysts. Several experiments have indicated that fish species may be able to be genetically manipulated in this way (Patil and Hong Woo 1996; Walker et al. 1995). However, these results await replication, and big questions remain over the effectiveness or otherwise of sperm cells as vectors *per se* (Smith 1999).

If it turns out that there is substance to claims that sperm cells can be induced to carry transgenes, then the techniques' efficiency would have to be high. Otherwise, electroporation of sperm cells could be as limited as it is (in principle) for zygotes, with excessively high numbers of animals needed in order to obtain each transgenic. The only way around this limitation would be via the development of a selection system for 'positive' sperm cells *in vitro* - a highly unlikely possibility, give that sperm cells exist in a quiescent state as far as gene expression is concerned.

Finally, it is worth noting that electroporation also has the potential to be used *in vivo*. This field is at a very early stage of development, but empirical improvements may in future permit electroporation to be used to deliver transgenes to particular tissues (Hofmann et al. 1999; Swartz et al. 2001). Given that there is no evidence that any particular somatic cell types are inherently unable to be successfully electroporated, it might in future become possible to direct *in vivo* electroporation to the spermatogenic (or sperm-storing) tissues, as an indirect route to the germline.

2.1.6 Naked DNA Auto-uptake

Empirical studies have shown that transgene constructs injected into muscle tissue or liver tissue *in vivo*, in the form of naked DNA molecules may be taken up and expressed by the muscle and liver cells. The same surprising effect has been obtained via intravascular injection of naked DNA. Cellular uptake of naked transgenes is believed to occur through microlesions within the cell membrane (Herweijer and Wolff 2003).

There have been no reports of naked DNA auto-uptake into zygotes, ESCs or cultured cells for NT. However, several reports of sperm cells associating with naked DNA have been published, as have reports of successful transgenesis in a broad range of animals following sperm auto-uptake of naked DNA. However, others have had difficulty replicating such work, and there are as yet no clear answers to the questions: (a) Is it possible; and (b) If so, how can it be reliably achieved? (Smith 1999). The

possibility that sperm cells may be able to pick up naked transgenes is discussed in more detail within the following section (2.1.7).

2.1.7 Sperm-mediated Gene Transfer

The scientific literature contains over seventy reports of the successful *in vitro* uptake of exogene constructs (transgenes) by animal sperm cells (Gandolfi 2000; Smith 1999; Smith and Spadafora 2005). A majority of these reports provide evidence of post-fertilisation transfer and maintenance of transgenes. Several of the studies report the subsequent generation of viable progeny animals, the cells of which contain transgene DNA sequences. While a minority of studies have used 'augmentation' techniques (electroporation or liposomes) to 'force' sperm to capture exogenes, the standard methodology is very straightforward: prior to *in vitro* fertilisation (IVF) or artificial insemination (AI), 'washed' sperm cells are simply incubated in a DNA-containing solution. As a potential tool for genetically manipulating animals, sperm-mediated gene transfer (SMGT) has the advantages of simplicity and cost-effectiveness, in contrast with more established methods of transgenesis such as pronuclear microinjection.

However, despite the above successes and regardless of its potential utility, SMGT has not yet become established as a reliable form of genetic modification. Concerted attempts to utilise SMGT have often produced negative results. The most notable example of such a failure is to be found in the collated results of several independent research groups: of 890 mice analysed, not a single animal contained transgene DNA (Brinster et al. 1989b).

Indeed, some biologists have expressed scepticism of the fundamental basis for SMGT (Birnstiel and Busslinger 1989; Chen and Chen 1996). Such scepticism is posited on the assumption that major evolutionary chaos would result if sperm cells were able to act as exogene vectors. Given that the reproductive tracts contain 'free' DNA molecules (originating from natural cell death and breakage), it seems

reasonable to expect sperm cells to be highly resistant to the risk of picking up such molecules (Smith 2002d).

Nevertheless, there exists a fairly well established body of empirical data showing that sperm cells are able, at least under particular experimental circumstances, to interact with and carry exogenes (Maione et al. 1998; Zani et al. 1995a). Furthermore, isolated reports of the successful use of SMGT for genetic modification continue to be published. A notable recent example is the generation of several transgenic pigs following the artificial insemination of sows with sperm cells preincubated with transgene DNA (Lavitrano et al. 1999; Lazzereschi et al. 2000).

There are two possible ways to make sense of the above experimental and theoretical considerations. The first possible explanation is that SMGT is fundamentally unattainable. If so, the empirical evidence in support of SMGT must be faulty. For example, perhaps sperm can associate with exogenous DNA but cannot convey the DNA into the oocyte; and transgene sequences may have been erroneously identified in tissue samples, perhaps due to DNA contamination affecting sensitive detection methods such as PCR. This scenario is certainly not impossible: scientific research contains several examples of theory being misled by mistaken data. Indeed, early reports of SMGT were compared with the (then contemporary) claims of "cold fusion" in physics (Birnstiel and Busslinger 1989). By contrast, the second possible explanation is that SMGT is viable, and that the claims of experimental success were not made in error. In this case, the explanation for the successful results must be that certain favourable factors applied in the fortuitous cases in which transgenes were taken up and transferred by sperm. Accordingly, several researchers have made efforts to elucidate such hidden parameters.

Underpinning such research into hidden factors has been the notion of the existence of 'inhibitory' factors (IFs) associated with sperm cells. These IFs are envisaged to prevent exogenous DNA uptake so as to protect the genetic integrity of the conceptus. The corollary of this notion is that successful instances of sperm cells taking up exogenous DNA may be attributed to the fortuitous removal or inhibition of IF(s) (Zani et al. 1995b).

Seminal fluid reportedly contains an inhibitory factor (IF-1) that appears to actively block the binding of exogenous DNA to sperm and to the above-mentioned proteins (Lavitrano et al. 1992). Additionally, three classes of proteins identified in sperm cells have been claimed to exhibit DNA-binding properties (Lavitrano et al. 1992; Lavitrano et al. 1997). There is also some evidence that the binding of transgene DNA can trigger the activation of endogenous nucleases in sperm cells, which cleave both transgene and sperm chromosomal DNA (Sotolongo et al. 2003; Spadafora 1998; Szczygiel et al. 2003). The possible existence of IF(s) or other mechanisms against foreign DNA may explain the varied and often negative results obtained from attempts to use sperm to act as transgene vectors.

A superficial binding of exogenous DNA to sperm cells would be very unlikely to result in successful transgenesis, given the rigours of fertilisation. Conceptually, therefore, it is necessary to envisage the exogenous DNA being actively taken up by the sperm cell. Ultrastructural autoradiographic studies have indicated that exogenous DNA becomes concentrated within the posterior part of the nuclear area of the head, the inference being that binding of DNA by the sperm is followed by internalisation (Camaioni et al. 1992; Francolini et al. 1993).

One very interesting possibility is the combination of naked DNA auto-uptake with microinjection, a process that has been termed 'transgenICSI'. In this recent approach, sperm exposed to naked transgene molecules are microinjected into oocytes. Success has been reported with mice, with approximately 20% of founder animals integrating and expressing the transgene (Perry et al. 1999; Szczygiel et al. 2003). Transgene uptake and expression following transgenICSI has also been reported in rhesus monkey embryos (Chan et al. 2000a; Chan et al. 2000b) and porcine embryos (Lai et al. 2001; Nagashima et al. 2003), although transgenic offspring did not result.

The success of transgenICSI provides support for the notion that sperm are indeed able to act as transgene vectors. However, some caution is required in making such a conclusion. Firstly, the experiments conducted need to be repeated and built upon before it can be said with certainty that the effect is a real one. Secondly, it could be the case that the transgene molecules bound only weakly to the sperm cells, such that only direct delivery (by ICSI) permitted the DNA to remain in place. If so, then this

would not support the notion that SMGT can work when used with IVF or AI, because weakly bound or superficially located DNA might be stripped away and lost from the incoming pronucleus during fertilisation. If it were correct that ICSI is an indispensable part of the process, then SMGT would appear to have little advantage over pronuclear microinjection in terms of inherent technical difficulties and expense. However, the efficiency of the process does appear to be somewhat better than that of pronuclear microinjection. The available experimental data on standard human ICSI (i.e. not involving genetic modification) indicate that: (a) the majority (ca. 75%) of eggs are successfully fertilised; and (b) lysis following ICSI occurs at a relatively low rate (ca. 10%) (Joris et al. 1998; Mansour 1998; Van Steirteghem et al. 1998). For transgenICSI, the reported rates of success (i.e. transgenics per transfer) vary, but a figure of around ca. 35% is fairly typical (Chan et al. 2000a; Chan et al. 2000b; Lai et al. 2001; Nagashima et al. 2003; Perry et al. 1999; Szczygiel et al. 2003).

Whereas it would be somewhat surprising if sperm cells have the inherent ability to easily capture and transfer naked transgene molecules such that the DNA remains in place during fertilisation, it remains conceptually possible to use transfection techniques to 'force' sperm cells to capture (and thus transfer) exogenous DNA. Success has been claimed in this regard using electroporation and liposome-mediated gene transfer. Since 1990, several reports claiming successful transgene uptake and/or transfer following electroporation of sperm cells have been published, and there have been a number of reports of sperm cells taking up liposome-encapsulated DNA (Smith 1999). More research is clearly needed to determine whether and to what extent transfection techniques such as liposome-mediated gene transfer or electroporation may be able to augment SMGT. Nevertheless, given that these gene transfer techniques have been shown to work with a wide range of somatic cell types, *in vitro* and *in vivo*, there is no reason to presume that sperm cells are inherently unable to be transfected using such methods.

An alternative possibility could be to introduce the transgene into testicular (sperm) stem cells *in vivo*. This would in principle remove the need to collect, manipulate or transfer eggs, thus providing a major streamlining of germline GM. Preliminary results have been reported in mice, where transgene constructs were directly injected into the testis. For example, 60-70% of sperm were reported to carry the transgene

following injection of naked DNA into the vas deferens (Huguet and Esponda 1998), with a follow-up report claiming detection of the transgene in the cells of 7.5% of offspring animals produced following fertilisation with the transgene-bearing sperm (Huguet and Esponda 2000). Similar results were reported by Sato et al, using liposome-encapsulated transgene molecules injected close to the epididymis (Sato et al. 1999a; Sato et al. 2002; Sato et al. 1999b).

In vitro gene delivery into *ex vivo* spermatogonial stem cells of both adult and immature animals has recently been reported (Brinster 2002). Nagano et al. obtained stable transgene integration and expression in up to 20% of murine spermatogonial stem cells following retroviral transgene delivery (Nagano et al. 2001). Genetically modified stem cells were transferred into the testes of infertile recipient mice, leading to transgeneity in ca. 4.5% of the resultant progeny, plus successful transmission to subsequent generations. Similar results were obtained by Orwig et al. in rats (Orwig et al. 2002). Although this form of transgenesis is at an early stage of development, preliminary work with spermatogonial stem cells in other mammals such as pigs and goats suggests that the approach is likely to be widely applicable (Honaramooz et al. 2003; Honaramooz et al. 2002). If human *ex vivo* spermatogonial stem cells are similarly able to pick up and transmit transgenes, an exciting potential route to germline gene therapy might emerge.

2.1.8 Combined Methods

It is possible to combine microinjection with one (or more) of the above transfection methods. A combination of microinjection with retroviral vectors has proved successful with bovines (Chan et al. 1998) and primates (Chan et al. 2001). In the primate case, microinjection was used to deliver a retroviral vector into the perivitelline space of 224 mature rhesus monkey oocytes (the oocytes were subsequently fertilized by intracytoplasmic sperm injection). The retroviral vector particles had an envelope type known to recognise and bind to the membrane of all cell types. The retroviral vector was microinjected at a developmental stage at which the oocyte nuclear membrane was absent, thus permitting nuclear entry. From 20

embryo transfers, three animals were born, one of which was transgenic. Additionally, a miscarried pair of twins was transgenic. Although this 'combined' method of gene transfer is laborious, it is the only approach that has permitted the generation of transgenic primates thus far.

One very interesting possibility is the combination of naked DNA auto-uptake with microinjection, a process that has been termed 'transgenICSI'. In this recent approach, sperm exposed to naked transgene molecules are microinjected into oocytes. Success has been reported with mice, with approximately 20% of founder animals integrating and expressing the transgene (Perry et al. 1999; Szczygiel et al. 2003). Transgene uptake and expression following transgenICSI has also been reported in rhesus monkey embryos (Chan et al. 2000a; Chan et al. 2000b) and porcine embryos (Lai et al. 2001; Nagashima et al. 2003), although transgenic offspring did not result.

Other delivery combinations that have been used to produce transgenic mammals include microinjection of bovine papilloma viral vectors (Mannik et al. 2003), microinjection of adenoviral vectors (Kubisch et al. 1997) and microinjection of transposable elements (Dupuy et al. 2002).

2.1.9 Novel Methods

Finally, it is worth considering a highly novel technique, as an illustration of the many and varied means by which emerging technologies are enabling gene transfer. In particle bombardment, DNA may be adsorbed onto spherical tungsten or gold particles (diameter c.4 μm) and transferred into a mass of cells by a particle gun; once inside the target cells, the DNA is solubilised and may be expressed (Pecorino 1995). This approach, sometimes known as 'biolistics', was originally developed for plant transgenesis but has been shown to be effective for transferring transgenes into mammalian cells *in vivo* (Cheng et al. 1993). Indeed, there are indications that biolistics may be more efficient than alternative methods such as liposome-mediated transfection and recombinant viral infection (Gainer et al. 1996), although the amount of research data presently available is too little to permit definitive comparisons. If the

method does prove to be effective *in vivo*, tumours are the most likely targets for particle bombardment (Mahvi et al. 1997).

Biolistics then, is a promising method for treating cells *en masse*, and looks most useful in terms of somatic gene therapy. There have been no reported attempts to utilise biolistics for altering zygotes. The *en masse* nature of the approach places it in a similar position to that of electroporation or liposome-based methods in respect of zygotes: impractically large numbers of zygotes would undoubtedly be required per successful transgenic event. However, in principle it might be possible to apply biolistics to ESCs or somatic cells for NT as a route to the germline.

2.2 Transgene Design

Transgenes are usually designed to express a particular gene product. In gene therapy, this would in principle be aimed at replacing a missing gene product in recessive 'loss of function' disorders. Alternatively, transgenes may be designed to eliminate an endogenous gene function. This would in principle be aimed at preventing an unwanted gene product causing damage in dominant 'gain of function' disorders.

2.2.1 Promoters

'Housekeeping gene' promoters, such as the β-actin promoter (Beddington et al. 1989) and the histone H4 promoter (Choi et al. 1991), can be fused with chosen structural genes. The 'housekeeping gene' promoters in such genetic constructs generally drive a fairly high level of constant transcription in most cell types and developmental stages when these constructs are integrated as transgenes.

Beyond simply driving gene expression, promoters may be chosen to allow specificity in, or control over, patterns of expression. A transgene comprising a particular structural gene fused with a tissue-specific promoter should only produce its gene product in the tissue(s) specified by that promoter. In terms of human germline gene

therapy, this might allow treatment to be directed exclusively to the required tissues or organs.

2.2.2 Control of Transgene Expression

If outside (i.e. experimenter) manipulation of gene expression is required, an inducible promoter may be used. Inducible promoters are able to respond to specific environmental cues such as temperature, or to dietary factors such as zinc. Thus the structural gene within a transgene can be switched on or off at will. For instance, a metallothionein (MT) promoter fused with a growth hormone (GH) gene (Palmiter et al. 1982) should allow GH production to be switched on simply by providing the transgenic with a zinc-supplemented diet. This might avoid the possible physiological difficulties associated with continuous global production - particularly *in utero* - of transgene products such as GH. Potential applications for inducible promoters in terms of gene therapy are conceivable.

More recently, inducible systems employing prokaryotic tetracycline resistance gene components have been developed (Gossen et al. 1995; Kistner et al. 1996; Park and RajBhandary 1998; Schultze et al. 1996; Shockett et al. 1995). These systems usually require two separate transgenes: thus, for use with transgenic animals (as opposed to cells *in vitro*) these systems usually require the establishment of two separate transgenic lines, each line containing one of the two transgenes. Double heterozygotes (containing both transgenes) are obtained by mating the two lines. One transgene (Transgene I) includes a promoter construct consisting of (a) an array of *tet* operator sequences and (b) a minimal promoter sequence; (a) and (b) are coupled to the gene that is to be controlled (Gene W). The other transgene (Transgene II) comprises a hybrid transcriptional transactivator gene fused to a suitable (e.g. tissue-specific) promoter. The hybrid transactivator gene product consists of a viral transcription-activating domain coupled with a tetracyline-binding domain. There are two main variants of the basic system: an 'on' system and an 'off' system. These variants are based upon functionally different transactivators. In the 'on' system, in cells in which Transgene II is active, exogenously administered tetracycline (or its analogue

doxycycline) binds to the transactivator protein: this renders the transactivator able to bind to the *tet* sequences on Transgene I, thereby activating expression of Gene W. By contrast, the transactivator in the 'off' system binds to the *tet* sequences only in the *absence* of tetracycline: thus, administration of tetracycline prevents expression of gene W.

Several other promoter-based systems for the control of transgene expression are in the developmental stage. Promising areas include natural promoters inducible by aryl hydrocarbons and promoter constructs inducible by steroid hormones (Fussenegger 2001; No et al. 1996; Saez et al. 1997; Smith et al. 1995; Wang et al. 1997; Wang et al. 1994).

Site-specific recombination provides a novel means of controlling transgene expression (Kuhn et al. 1995; Stark et al. 1992; Utomo et al. 1999). As with the tetracycline approach (above), two separate transgenes are usually required, necessitating the mating together of separate transgenic lines to produce double heterozygotes (where transgenic animals are required). One transgene (Transgene I) consists of an appropriate (e.g. tissue-specific) promoter coupled to the gene that is to be controlled (Gene X), engineered to contain a strong stop signal flanked on each side by a recombinase recognition site (e.g. *loxP* from bacteriophage P1). The other transgene (Transgene II) consists of a recombinase gene (*Cre* in the case of bacteriophage P1) fused to an inducible promoter. Exogenous administration of inducer drives the production of Cre recombinase from Transgene II. The Cre recombinase binds to the *loxP* sites on Transgene I and catalyses the excision of the flanked stop signal, thereby rendering Gene X competent for expression. A variant of this system can be used to *inactivate* a transgene, in which Gene X (or an essential component thereof) is flanked by recombinase recognition sites. In this case, recombinase production results in the removal of essential sequences, thereby eliminating expression of gene X.

2.2.3 Episomal Vectors

Various extrachromosomal plasmid vectors (episomes) have been used as transgenes (Colosimo et al. 2002; Stoll and Calos 2002). Such vectors have been employed to produce transgenic animals, via a variety of routes including pronuclear microinjection and SMGT (Celebi et al. 2002; Khoo et al. 1992; Mannik et al. 2003). However, episomal plasmid vectors tend to behave in an unstable fashion in transgenic animals (Celebi et al. 2002; Mannik et al. 2002). During development, plasmid copy numbers fluctuate and plasmids are lost from some cells. Plasmid inheritance to subsequent generations of animals is similarly problematic. Moreover, worrying health problems (such as tumour formation) have been associated with some episomal vectors (Lacey et al. 1986). Of course, the behaviour of an episome must relate in large part to its genetic constitution, and therefore stability problems and safety limitations may in principle be surmounted by improved plasmid design.

Autonomous artificial 'mini-chromosomes', (mammalian artificial chromosomes, MACs) have been constructed and successfully introduced into mammalian cells (de Jong et al. 2001; Grimes and Cooke 1998). MACs comprise centromeres, telomeres and replication origins, and are maintained autonomously within the host cell. Structural genes, promoters and enhancers (etc) can be included in MACs. Preliminary research indicates that MACs can be used, via pronuclear microinjection, to create transgenic animals in which the MACs are maintained autonomously (Co et al. 2000).

2.3 Random Integration of Transgenes

Transgenes usually integrate into a random position within the host genome. The arrangements of exogenous DNA integrated into the chromosomes of cultured mammalian somatic cells are very similar to the arrangements found in transgenic animals (Brinster et al. 1985; Folger et al. 1982; Gordon and Ruddle 1985). This is true regardless of whether the transgenic animals have been derived from one-cell

embryos or ESCs. This suggests that the underlying molecular mechanisms of random integration are essentially the same in all cell types.

The main features of randomly integrated exogenous DNA are as follows:

- Integration occurs at a frequency of between 10 - 30% of cells in which transgene DNA is delivered to the nucleus;
- Integration occurs at one or, rarely, a few chromosomal sites per nucleus;
- Integrated DNA is usually present in the form of a multicopy array;
- The vast majority of arrays consist of head-to-tail associations.

The precise molecular mechanisms of random integration are not known. However, experimental data such as that outlined above has allowed the construction of models of random integration. A general model is presented below.

2.3.1 Concatenation

The fact that transgenes are usually present as arrays (concatemers) indicates that extrachromosomal events (concatenation) take place prior to chromosomal integration.

Random end-to-end joining (ligation) of transgene molecules should generate head-to-tail, head-to-head and tail-to-tail associations in a ratio of 2:1:1. However, as mentioned above (Section 2.3), the vast majority of arrays take the form of head-to-tail associations. Therefore, end-to-end joining cannot be an adequate explanation of concatenation. However, rare head-to-head/tail-to-tail associations do occur, so ligation would appear to be a possibility. The simplest explanation lies in the kinetics of free transgene molecules: it must be stochastically infrequent for any two transgene molecules to meet together in an end-to-end fashion, and stay together long enough for a molecule of DNA ligase to unite them (Bishop 1996).

If end-to-end joining cannot explain the majority of concatenation events, another form of interaction between transgene molecules must be operative. The most likely process is extrachromosomal HR between circular and linear molecules. This mechanism can be shown, in formal geometric terms, to generate exclusively head-to-tail concatemers (Bishop 1996).

A prerequisite for concatenation by extrachromosomal HR is the co-existence of both circular and linear transgene molecules. Experimental data shows that head-to tail arrays result (with equal frequency) following the introduction of either circular or linear molecules (Brinster et al. 1985; Folger et al. 1982). Therefore it is necessary to postulate the existence of two nuclear processes: (1) circularisation (by ligation of the free ends of a transgene molecule); and (2) linearisation (by random nuclease action).

Linearisation would generate circularly permuted molecules. HR could then occur between circularly permuted and circular molecules, or between circularly permuted and input linear molecules, or between individual (different) circularly permuted molecules: in all cases the effect would be the formation of a head-to-tail concatemer. Repeated rounds of HR would extend the array (i.e. increase the number of transgene copies therein).

Several cultured cell studies have demonstrated that linear DNA molecules are circularised by intracellular ligation, and a number of similar studies have indicated that circular DNA molecules are randomly cleaved (Bishop and Smith 1989). Thus, circularly permuted molecules will be produced following introduction of exogenous DNA molecules.

Concatenation of circularly permuted molecules can most simply be explained by the following events: (1) homology pairing; (2) exposure of single-strand substrates at the end of each duplex; (3) formation of a duplex between exposed complementary strands; and (4) resolution (by repair of the duplex).

The fact that extrachromosomal HR occurs with high frequency amongst individual transgene molecules contrasts sharply with the low frequency of HR between transgenes and homologous chromosomal sequences. There would appear to be two

possible explanations, as follows: (a) the 'free' nature of the interacting transgene molecules in some way enables HR to proceed very efficiently; and/or (b) the free (non-telomeric) ends of the interacting transgene molecules are very good substrates for recombinase enzyme activities. However, the molecular/biochemical details of HR remain to be elucidated; thus it is not yet possible to give a precise explanation for the contrast that exists between extrachromosomal HR and the HR that underlies gene targeting.

2.3.2 Illegitimate Recombination

Transgene or transgene array integration only occurs in a minority of surviving transfected cells, suggesting that (nontargeted) integration is the result of a rare intranuclear event. The simplest model would suppose that the rare intranuclear event is chromosomal (double-strand) breakage followed by end-joining between the transgene ends and the chromosomal broken ends. Certainly, the frequency of DNA integration is increased by irradiation of the transfected cells (Perez et al. 1985).

An alternative mode of chromosomal integration could be 'illegitimate' recombination between very poorly matched sequences. Studies of the nucleotide sequences at exogenous-endogenous DNA junctions have shown illegitimate recombination in a number of cases, although the overall number of studies is small (Bishop 1996).

Interestingly, a diverse range of chromosomal sequence disturbances has been found in junctional studies. These include, in order of frequency: deletions, duplications, inversions and more complex rearrangements including the appearance of sequences from elsewhere in the genome or even of unknown origin. Bishop (Bishop 1996) has proposed an explanation of these observations that sees exposed single-stranded ends of transgene molecules initiating recombination by invading DNA duplexes.

It is now known that the majority of transgenic founder animals are mosaics. This suggests that integration occurs during DNA replication. Wilkie & Palmiter (Wilkie

and Palmiter 1987) have proposed that the free ends of the transgene initiate recombination by invading a replication 'eye'.

It may be that more than one integration route is possible for randomly integrated transgenes. A full understanding of the mechanisms of random transgene integration awaits further study at the molecular/biochemical level. Such understanding is important from the perspective of gene targeting, because it may be that targeting frequencies could be enhanced by somehow blocking 'background' (i.e. random) integration events.

2.3.3 Problems Associated with Random Transgene Integration

Random integration poses the risk of insertional mutagenesis, where a transgene integrates into or close to an important endogenous gene. Although this is a real risk, in practice transgenic animals rarely suffer from insertional mutagenesis. This is partly because 'important' genes are often most important during development: insertional inactivation of such a gene at the start of development (i.e. at the time when germline gene transfer occurs) would simply lead to embryonic death. For other, less crucial genes, haplosufficiency may counterbalance insertional inactivation. And the probability of a transgene inserting randomly into an endogenous gene is relatively low for each integration event, given the fact that genes represent only a minority of the sequences within the mammalian genome. Nevertheless, insertional mutagenesis has on occasion been observed to impact upon the health of transgenic animals: physiological, behavioural and oncogenic effects have been noted.

A second problem for randomly integrated transgenes concerns transgene expression. Randomly integrated transgenes tend to give poor levels of expression, or exhibit inappropriate expression, in the form of temporally or spacially (ectopic) aberrant expression. Moreover, random integration leads to inconsistent outcomes: individual transgenic animals produced using an identical approach (i.e. same transgene, same

transfection method, same genetic background of host animal) differ greatly from each other in terms of transgene expression.

There are three main reasons for such problems with expression. Firstly, the particular genetic environment at any point of insertion is likely to influence the expression of the integrated transgene, a phenomenon known as the 'position effect'. Secondly, the multicopy transgene arrays that predominate in random integration outcomes exhibit a paradoxical expression pattern: the larger the array, the poorer the level of expression. The number of transgene copies within an array is thought to positively correlate with the degree to which the array will be methylated by the host cell. Finally, the high level of mosaicism associated with random integration leads to expression difficulties: generally low expression results from the absence of transgene in all tissues and, given that patterns of mosaicism are themselves random, expression is variable and inconsistent between individual transgenics.

Approaches to circumvent or mitigate expression difficulties exist or are emerging. In some cases the remedy lies with transgene design: for example by ensuring that an appropriate enhancer sequence is included in the transgene construct. Beyond this, it may be possible to insulate a gene from the position effect. Matrix attachment regions (MARs) are sequences which, when placed on either side of a gene within a transgene construct, appear to allow the gene(s) within an integrated transgene to occupy a separate chromosomal domain. Locus control regions (LCR) have a similar effect. However, results with transgenic animals have been variable, and the extent to which 'insulator' sequences may be able to avoid the position effect remains to be determined.

It is clear that random integration of transgenes is associated with serious drawbacks. Although the risk of insertional mutagenesis is low in the case of animal transgenesis, the same level of risk in human germline gene therapy would present more of a problem. Even if the risk was deemed acceptable (perhaps in the case of gene therapy for a very serious candidate disorder), the problem of unreliable and inconsistent expression associated with random integration would remain, unless significant advances in transgene design are forthcoming.

Ultimately, the best solution to aberrant transgene expression and insertional mutagenesis would be to avoid the problems associated with random integration entirely. This is achievable through gene targeting: a transgene targeted to a chosen genomic locus will by definition avoid the position effect, it will normally be present as a single copy rather than as a multicopy array, and mosaicism will not normally be a problem. Moreover, gene targeting can be used to precisely alter endogenous genetic sequences, such that problematic (i.e. disease-causing) endogenous genes can be 'knocked-out', or even repaired. Thus, gene targeting would be of the very greatest importance in human germline gene therapy. Gene targeting is the topic of the next chapter.

Chapter 3: Gene Targeting

Gene targeting may be defined as the directed genetic modification of a chosen endogenous genomic locus. Gene targeting is an achievable goal in mammalian cells. However, progress has been limited by a lack of targeting efficiency. Studies on mammalian cells *in vitro* demonstrate that the vast majority of interactions between transgene and endogenous DNA result in random rather than targeted integrations. The reported ratio of random to targeted integration varies enormously, from around 1:4 to more than 1,000,000:1. In most cases, the ratio is between 1000:1 and 10,000:1 (Bollag et al. 1989; Capecchi 1989; Frohman and Martin 1989; Sedivy and Dutriaux 1999; Thomas et al. 1992; Vasquez et al. 2001). It is noteworthy that the low efficiency of targeted integration in mammalian cells is in marked contrast to that which occurs when DNA is transfected into lower eukaryotes such as yeast: under appropriate empirical conditions, targeting is the norm and random integration the exception for such organisms.

3.1 Cell Types and Gene Targeting

Due to the low efficiency of targeting, it is normally necessary to select for targeted outcomes against a background of random outcomes. Such selection can only be applied to somatic cells growing and dividing *in vitro*: thus, selection-based gene targeting is not applicable with zygotes or sperm cells. However, methods have been developed whereby cultured cells can, following selection for gene targeting, be used to produce transgenic animals.

3.1.1 Zygotes

Targeted outcomes cannot be selected for in zygotes. This does not, however, mean that targeted events are impossible in zygotes. Very few studies have looked at gene targeting in zygotes, probably due to the expense of gene transfer and analysis. A landmark study by Brinster et al. (Brinster et al. 1989a) involved the analysis of 506 transgenic founder mice. These animals were produced by microinjecting zygotes

from mice containing a deletion in the major histocompatibility (MHC) class II *Eα* gene. The transgene construct was based on sequences from this gene and included the sequences absent in the host mice. A single mouse was found to have undergone targeted correction of the *Eα* gene deletion. This study shows that gene targeting is possible in zygotes. Although it is not possible to determine an accurate frequency of gene targeting in zygotes from this work, it appears that the rate of targeting (1 in 506 animals), although quite high compared with cultured cells (see above), is too low to permit the efficient use of gene targeting in zygotes.

3.1.2 Embryonic Stem Cells

Inner cell mass (ICM) cells from the mouse blastocyst can be propagated *in vitro* as embryonic stem cells (ESCs) (Abbondanzo et al. 1993; Brook and Gardner 1997). In contrast to other cultured cell lines, ESCs retain their normal karyotype even after many months in culture, during which time they remain totipotent (able to contribute to both somatic and germ lines). Furthermore, ESCs are capable of colonising the embryo. These unique properties allow ESCs to form chimeras when injected into blastocysts or aggregated with morulae. The resultant embryos can be transferred to the uterus of a pseudopregnant female mouse for gestation. In cases where an ESC has successfully contributed to the embryo, the resultant offspring will be chimeric (up to ca. 50% of animals). The ESC contribution to a mouse can high (up to ca. 80% of the cells), and will often include the germline cells. However, it should be noted that, with some transgenes, and for reasons that are not understood, the production of chimeras can be problematic or even unattainable, especially when germline transmission is required to breed pure lines of heterozygous or homozygous animals (Abbondanzo et al. 1993; Brook and Gardner 1997; Robertson 1987; Torres 1998).

It is during the *in vitro* culture stage that ESCs may be transgenically manipulated (Pirity et al. 1998; Torres 1998). Many gene delivery systems are effective with ESCs, including viral vectors, liposomes, and electroporation. The great advantage of ESCs is that they can be subjected to a range of selective agents *in vitro,* which allows the selection of particular transgenic modifications. This ability makes ESCs extremely

useful for gene targeting experiments and applications (Metzger and Feil 1999; Muller 1999).

However, the use of ESCs is limited because, to date, the mouse is the only animal from which ESC lines have been unequivocally established. It would be surprising if this limitation represents a fundamental biological barrier. However, further empirical work is needed before totipotent ESC lines become available for other species. Indeed, efforts to isolate non-murine ESCs have been ongoing for nearly two decades but to date no germline-competent ESCs have been isolated in other vertebrates (Prelle et al. 1999; Wheeler et al. 2003).

3.1.3 Nuclear Transfer

The successful transfer of 'reprogrammed' sheep donor nuclei has recently been achieved (Campbell et al. 1996; Schnieke et al. 1997; Wilmut 1997). Unfertilised, metaphase-stage enucleated ('universal recipient') eggs received the transferred nuclei. Donor nuclei originated from somatic cells that had been forced into a form of cell cycle stasis (by incubating the cells in a minimal nutrient medium), such that DNA replication and gene expression were halted (or virtually so). Nuclear transfer was conducted by depositing a donor cell under the zona pellucida of a universal recipient egg, and fusing the two cells by electrical stimulation. This process resulted (in some cases) in successful embryo development, the donor nuclei having been 'reprogrammed' into totipotency. Offspring were produced following the transfer of such 'reconstructed' embryos to recipient ewes. Subsequent molecular genetic testing showed that the lambs' DNA had originated from the donor cells. In some of the experiments, the donor nuclei were obtained from embryo-derived cultured cell lines. Following these groundbreaking experiments, successful cloning from cultured cells of various mammals including cattle, goats and pigs has been reported (Tsunoda and Kato 2000; Wolf et al. 2000). Interestingly, a human ESC line has recently been derived from cloned human blastocysts produced by NT (Hwang et al. 2004), pointing to a possible new field of application for NT technology.

The prospects for germline GM via NT are very significant: transgenes can be introduced to somatic donor cells *in vitro,* permitting germline genetic modifications. This has been achieved in animals such as sheep (Schnieke et al. 1997). Several gene delivery systems are applicable to NT donor cells, including liposomes and electroporation. Moreover, because selection can be applied to cultured donor cells, NT can be used to achieve germline gene-targeting. Gene targeted transgenic animals have been created in this way (Clark et al. 2000; McCreath et al. 2000). Thus, NT is potentially able to provide the same range of transgenic manipulations presently available in mice (via the ESC route) to all mammal species.

However, in comparison with ESC transgenesis, NT has thus far proved to be relatively inefficient: only a small proportion of reconstructed embryos survive to become live animals. For example, McCreath et al. produced live targeted sheep at an efficiency of less than 4% (McCreath et al. 2000), and Lai et al. produced live targeted pigs at an efficiency of less than 2% (Lai et al. 2002b).

The health status of NT-derived animals is also proving to be problematic (Renard et al. 2002; Smith et al. 2000). Developmental abnormalities are very common, with a high birth weight being the predominant feature, a phenomenon known as "large offspring syndrome" (LOS). The abnormalities frequently result in death (foetal or postnatal) or debility. For example, of fourteen live-born lambs, seven died within 30 hours of birth, and four died within twelve weeks (McCreath et al. 2000). Similarly, out of seven piglets, two piglets died shortly after birth, and one died at 17 days; only one appeared to be entirely free of developmental abnormalities (Lai et al. 2002b). Transgenesis and gene targeting are not of themselves implicated: the health problems are associated with NT *per se.* During the *in vitro* (cell culture) stage, the pattern of chromosomal imprinting may change; there are indications that inappropriate expression of imprinted genes following such epigenetic alteration may be mainly responsible for the poor health of NT-derived animals (Kono 1998; Rideout et al. 2001; Wakayama and Yanagimachi 2001). Research into epigenetic reprogramming in NT embryos is in progress, and it is to be hoped that developmental abnormalities arising from NT will eventually be eliminated or reduced in frequency. Meanwhile, it is anticipated that NT-related health problems, to the extent that the basis for such is epigenetic, are unlikely to affect the offspring of surviving first-generation animals.

3.1.4 Non-selective Gene Targeting

In embryonic stem cells and in certain somatic cells *in vitro*, unusually high levels of gene targeting have been reported. Isogenic transgenes, derived from the same (syngenic) laboratory animal strain as the target animal, contain homology blocks that are genetically identical (or virtually identical) to the target homology regions. Riele et al. (Riele et al. 1992) reported a 20-fold improvement in targeting efficiency when an isogenic transgene was used to target the retinoblastoma susceptibly gene (*Rb*) in murine ESCs, yielding a remarkably favourable ratio of random to targeted integration (approximately 1:4). Similar results were obtained from a systematic study by Van Deursen and Wieringa (Van Deursen and Wieringa 1992), in which the creatine kinase M gene (*CKM*) in ESCs was targeted.

More recently, adeno-associated virus (AAV) vectors have been used to gene target somatic cells at high frequencies. Hirata et al. used AAV vectors to introduce transgenes into the hypoxanthine phosphoribosyl transferase (*HPRT*) and Type I collagen (*COL1A1*) loci in normal human fibroblasts (Hirata et al. 2002). The transgenes were targeted at high frequencies, such that the majority of transgene-containing cells had undergone gene targeting with an appropriately designed vector. AAV targeting frequencies have been further improved by selective creation of double-strand DNA breaks in the target site (Miller et al. 2003; Porteus et al. 2003). Most recently, adult human mesenchymal stem cells (MSCs) have also been targeted with high efficiency using AVV vectors (Chamberlain et al. 2004).

3.2 Targeted Integration

The utility of gene targeting as a means of gene therapy requires systematic study into the mechanisms of the process. Given the many variables involved in any gene targeting experiment (i.e. cell type, transfection method, transgene design, target site,

etc.), it is unsurprising that progress towards a detailed understanding has been relatively slow.

Nevertheless, various studies have provided important insights into several aspects of mammalian gene targeting. Initial studies involved the use of artificially introduced selectable target sites in mammalian cell lines, such that rare targeting events could be recovered. Later studies have used targets of natural loci in mammalian cell lines, ESCs and mammalian zygotes. Various observations and inferences from such studies are considered in the following sections (3.2.1 - 3.2.8).

3.2.1 Transfection Method

In methods other than microinjection, the exogenous DNA molecules must somehow migrate through the cytoplasm of the host cell in order to reach the nucleus. Of the DNA that survives this journey, a substantial proportion sustains some degree of endonucleolytic or exonucleolytic damage. In contrast, virtually no damage occurs to DNA delivered directly into the nucleus by microinjection (Lebkowski et al. 1984; Wake et al. 1984).

Gene targeting using a damaged transgene is unlikely to be desirable in gene therapy. Beyond this concern, it may be the case that damage sustained by incoming transgene molecules renders them less able to undergo HR. The reasons for this are not known, but the effect seems to exist. For example, separate studies were conducted by Lin et al. (Lin et al. 1985) and Thomas et al. (Thomas et al. 1986) both involving gene targeting of artificially introduced defective genes in mouse fibroblasts. Lin et al. reported a ratio of random integration to gene targeting of 100,000:1 whereas Thomas et al. reported a ratio of 100:1. The major difference between the two sets of studies lies in the method of transfection, with Lin et al. using calcium phosphate co-precipitation, and Thomas et al. using microinjection. Similarly, in a systematic study using the adenine phosphoribosyltransferase (*APRT*) locus in Chinese hamster ovary cells, Vasquez et al. (Vasquez et al. 2001) compared the targeting efficiencies associated with various transfection methods. In these experiments, mass-delivery

methods (elecroporation, co-precipitation, liposomes) yielded an average ratio of random integration to gene targeting of 200,000:1 (range 2,400:1 to 350,000:1) compared with a ratio of 1:15 for microinjection. In addition to the possibility that HR frequency is reduced due to DNA damage associated with the mass-transfection methods, it has been suggested that the larger numbers of transgene molecules delivered by the mass-transfection methods may overwhelm the HR machinery (Vasquez et al. 2001).

3.2.2 Transgene Sequences

Gene targeting is dependent on HR, which is in turn dependent on shared homology between recombining DNA sequences. The question is, how much homology is required for optimal efficiency of gene targeting? There is at present no complete answer to this question, because systematic studies are lacking, and comparison between separate studies is very problematic due to the existence of several variables other than the extent of homology. Such variables include other transgene sequences, the physical state of the transgene, the cell type used, the target gene and the actual nature (rather than simply the extent) of homology. Nevertheless, several studies have provided a partial answer. Thomas and Capecchi (Thomas and Capecchi 1987), targeting the *HPRT* gene in ESCs, found that targeting efficiency appeared to be strongly dependent upon the degree of homology possessed by the transgene (and shared with the target locus). Specifically, an increase in homology from 4 kb to 9.1 kb correlated with 40-fold increase in the rate of targeting, as measured by the ratio of targeted:random integration. Shulman et al. (Shulman et al. 1990), targeting an immunoglobulin gene in hybridoma cell lines, varied the extent of homology from 1.2 to 9.5 kb. Again the degree of homology correlated with targeting efficiency, with a 25-fold increase seen over the range from 2.5 to 9.5 kb. No increase in targeting efficiency was observed between 1.2 and 2.5 kb. From these studies it can be concluded that the frequency of gene targeting is roughly proportional to the extent of homology shared by the transgene and its target locus. However, it is notable that the effects of very large (> 9.5 kb) homologies are not known.

Besides homology length, base pair variation may affect the rate of targeting. This is evident from experiments comparing isogenic and nonisogenic transgenes. The homology region(s) in an isogenic transgene is derived from the same (syngenic) laboratory animal strain as the target animal. Therefore, isogenic transgenes contain homology blocks that are genetically identical (or virtually identical) to the target homology regions. By contrast, a homology stretch in a nonisogenic transgene will typically be interrupted by a number of slight sequence divergences, such as base-pair mismatches and small deletions / insertions. In a series of experiments designed to compare isogenic and nonisogenic transgenes, Riele et al. (Riele et al. 1992) reported a 20-fold improvement in targeting efficiency when an isogenic transgene was used, yielding a remarkably favourable ratio of random to targeted integration (approximately 1:4). The target site was the retinoblastoma susceptibly gene (*Rb*) in an ESC line derived from mouse strain 129. The isogenic and nonisogenic transgene constructs contained 17 kilobases of homology, derived respectively from (a) mouse strain 129 and (b) mouse strain BALB/c. Similar results were obtained from a systematic study by Van Deursen and Wieringa (Van Deursen and Wieringa 1992), in which the creatine kinase M gene (*CKM*) in ESCs was targeted with transgenes sharing 9 kb of homology with the target site. In these experiments, an increase in targeting efficiency of approximately 25-fold was observed when isogenic transgenes were used. Thus, the use of isogenic DNA in transgenes appears to hold promise for improving gene-targeting efficiencies. However, it has not been established whether the outcomes described above are applicable to other target genes in other cell types. Moreover, there exists a dearth of systematic knowledge concerning the nature, frequency and extent of heterologies that may affect targeting efficiencies. Nevertheless, it is reasonable to conclude that, all other factors being equal, transgenes employing perfect homology are likely to yield better targeting efficiencies in comparison with those using interrupted homology.

3.2.3 Physical State of the Transgene

In keeping with yeast data (see Section 3.2.8), all studies agree that linearization of the transgene (in the region of homology) greatly enhances targeting efficiency

(Kucherlapati et al. 1984). This finding is supportive of the double-strand break-repair model as an explanation of the mechanism of gene targeting (Smith 2001). Beyond linearization, stripping the transgene ends to expose around 200 nucleotides of single-stranded DNA appears to further enhance targeting (Vasquez et al. 2001). Although the underlying mechanism is not understood, the finding that single-stranded transgene tails enhance targeting fits well with the notion that HR involves single-stranded DNA ends invading target duplex DNA (Sun et al. 1991).

3.2.4 Transgene Copy Number

A targeting transgene molecule presumably has to 'search' through the host genome until it 'finds' its target sequence. Therefore, it might be expected that targeting efficiency would be enhanced by increasing the number of transgene molecules introduced to the host cell. An increasing cytotoxic effect is observed where increasingly large quantities of DNA are microinjected into the nucleus (Hogan et al, 1994). However, the targeting efficiency can still be obtained, by calculating the proportion of surviving cells that have been successfully targeted. No study has demonstrated a correlation between transgene copy number and targeting frequency. This area has not been extensively researched, but at least two studies have positively determined that there appears to be no relationship whatsoever between the number of copies introduced and the efficiency of gene targeting (Rommerskirch et al. 1988; Thomas et al. 1986). The inference must be that the initial search for homology does not seem to be the rate-limiting step for targeting. This conclusion is also supported by experiments involving amplification of the target site (see Section 3.2.8): an increased target copy number does not appear to enhance the frequency of targeting. Indeed, if Vasquez et al. (Vasquez et al. 2001) are correct in postulating that too many transgene molecules may overwhelm the HR machinery (see Section 3.2.1 above), it may turn out to be the case that an inverse relationship exists between transgene copy number and targeting efficiency.

3.2.5 Position of Target Site

The position of the target site within the genome does not strongly influence the frequency of HR. For example, twelve independent recipient cell lines were produced by Thomas et al. (Thomas et al. 1986), each line containing a defective neomycin-resistance gene integrated at a different chromosomal position. Introduction of targeting DNA constructs gave similar gene targeting frequencies in all twelve lines.

3.2.6 Recombination Hotspots

Targeting the endogenous β_2-microglobulin gene in ESCs, Zijlstra et al. (Zijlstra et al. 1989) achieved a very high frequency of gene targeting (a ratio of about 1: 25 targeting to random integration). Other investigators for the β_2-microglobulin target gene (Koller and Smithies 1989) and for the *Hox 3.1* target gene (Lemouellic et al. 1990) have reported similarly high targeting frequencies. Such studies support the existence of recombination 'hotspots'. However, the sequences involved in such hotspots remain to be elucidated.

3.2.7 Target Gene Activity

There is no evidence that the level of expression of the target gene correlates with the frequency of gene targeting (Koller and Smithies 1989).

3.2.8 Target Copy Number

As noted in Section 3.2.4, experimental amplification of the target site does not appear to enhance the frequency of targeting. For example, Zheng and Wilson (Zheng and Wilson 1990) used two mammalian cell lines, one of which contained 2 target gene copies. The second line contained around 800 target gene copies, located in three

clusters on different chromosomes. Gene targeting rates were the same in both cell lines. Similarly, Thomas et al. (Thomas et al. 1986) used three cell lines containing integrated target plasmid sequences present as one copy, four dispersed copies or five tandem copies. Again, the rates of gene targeting were similar in all three lines. Such results infer, as suggested previously (Section 3.2.4), that the initial search for homology does not appear to be the rate-limiting step for targeting. Interestingly, some target amplification experiments in yeast have suggested that the frequency of gene targeting does depend on the number of target copies. Indeed, Wilson et al. (Wilson et al. 1994) report a linear relationship between target site copy number and the rate of targeting in yeast. The reason for this difference between yeast cells and mammalian cells remains to be established.

3.3 Concluding Remarks

Although major progress in model building has been made in recent decades, extensive biochemical analysis of the molecular mechanism(s) of HR will be required if gene targeting is to be better understood. On a different level, the phenomenology of gene targeting also requires extensive systematic analysis.

Absolute frequencies of gene targeting in mammalian cells remain low, and the ratio of targeted to random integration is still heavily weighted in favour of the latter. Until the frequencies are improved, the potential use of gene targeting in non-selective systems will be limited. Such improvement is likely to depend upon a more detailed understanding of gene targeting, which is in turn dependent upon systematic analysis of the sort described above.

Chapter 4: Potential Applicability of Genetic Modification Technology to the Human Germline

4.1 Introduction

The previous chapter introduced and discussed the major transfection methods, transgene design principles and forms of transgene integration involved in the production of transgenic animals. The purpose of the present chapter is to consider how such technology might be used to achieve human germline GM.

4.2 Criteria for Assessing Applicability to Human Germline Gene Therapy

An ideal gene transfer system in the context of human germline gene therapy would have the following features: (a) the ability to deliver transgenes in a highly efficient manner; (b) non-prohibitive cost and expertise requirements; (c) minimal risk of causing insertional DNA damage; (d) low rate of mosaicism; (e) high DNA carrying capacity; (f) the ability to permit adequate and controlled transgene expression; and (g) the ability to target transgenes to precise genomic loci.

Unfortunately, no single system amongst the presently available systems is able to provide all of features (a-g) above. Indeed, some gene transfer systems are so thoroughly unsuited to human germline gene therapy that they are not considered here. Of the systems that offer some positive features, in every case major drawbacks exist. In each case, particular scientific advances are required before the methods would be suitable for use in human germline gene therapy. In this respect, methods that require a relatively small degree of scientific research should be seen as more plausible than methods requiring many years of progress towards distant – possibly unobtainable – goals (Smith 2004).

4.3 Gene Transfer to Human Embryos

Most transgenic animals have been produced via the introduction of transgenes into embryos, and the associated technology and underpinning science is accordingly well developed. Thus, the human embryo is a potential candidate for human germline gene therapy.

Unless dramatic improvements in the technologies are forthcoming, certain transfection methods are not at present a realistic proposition for gene transfer into human zygotes. Such methods include liposome-mediated gene transfer, electroporation, naked DNA uptake and many viral vectors. The problem is one of low transfection frequencies, coupled with fact that zygotes must be harvested (as opposed to grown *in vitro*). This leaves pronuclear microinjection and retroviral transfer as the only contenders presently available that might be adapted for use with embryos in human germline gene therapy.

4.3.1 Pronuclear Microinjection

Pronuclear microinjection would be an obvious choice of transgene delivery method for human embryos. The technique is well established in animals, and is likely to be directly applicable to the human zygote (Houdebine 2002; Wall 1996). Zygotes from various mammalian species have particular characteristics that necessitate amendments to the basic (murine) technique. For example, bovine and porcine zygotes are optically opaque, due to the presence of lipid granules in the cytoplasm; this necessitates centrifugation to displace the obscuring cytoplasmic material such that the pronuclei become visible. Similarly, the pronuclei in ovine zygotes are very difficult to visualise, due to sharing a very similar refractive index with the cytoplasm; this necessitates the use of top-quality optics, such as differentiation interference contrast (DIC) microscopy, instead of standard phase contrast microscopy. Thus, empirical adjustments enable pronuclear microinjection to be employed with zygotes from essentially any mammal. It would be surprising and unfortunate if the human

zygote proved to be an exception to this rule. Indeed, visualisation of the pronuclei in human fertilised eggs is not problematic.

Although pronuclear microinjection would probably be usable with human zygotes, the major inherent problems of the method render it less than ideal for human germline gene therapy. A major problem is the relatively low rate of transgene integration: in mice, the overall efficiency of transgenesis (taking into account embryo loss *in vitro* and *in vivo*) is typically ca. 2% (Bagis and Papuccuoglu 1997; Hirabayashi et al. 2001; Page et al. 1995). This level of efficiency is perfectly practicable for animal transgenesis, but it would be problematic for humans. Moreover, murine pronuclear microinjection transgene uptake values are several times higher than those achieved with other (non-rodent) species. Accordingly, even with hormonal induction of superovulation, the numbers of zygotes available per woman would be a strongly limiting factor in the potential use of pronuclear microinjection for human germline gene therapy.

Embryo pre-screening (preimplantation genetic screening) might be one possible way around the problem of low transgene uptake efficiency. Using established techniques, one or two blastomeres could be taken from 8 cell stage embryos and analysed by PCR for the presence of transgene DNA. However, such pre-screening would not be 100% reliable, due to mosaicism within the early embryo. Following microinjection and successful integration of the transgene sequences, the transgene would be expected to be present in only 50% of the resulting blastomeres. Assuming that in humans, as with mice, 3 blastomeres are recruited to form the entire inner cell mass (ICM) (Bishop 1999; Gilbert 1997), then 1 in 8 of the resulting individuals would contain no transgene sequences, another 1 in 8 would contain transgene sequences in 100% of their cells, and the remaining 6 from 8 individuals would be mosaics, consisting of $1/3^{rd}$ or $2/3^{rd}$ transgene-containing cells. Accordingly, pre-screening would have a failure rate of more than 50%. The only feasible way that pre-screening might work at an acceptable level of efficiency would be to screen blastocyst-stage embryos. However, blastocyst biopsy techniques are in their infancy, and it remains to be seen whether such techniques could be applied to ICM cells (as opposed to trophoblast cells).

Extrapolating from murine data, it would typically require ca. 50 zygotes to produce one genetically modified individual. Assuming 8 eggs per superovulation cycle, it would take approximately 6 months per woman to obtain 50 eggs. Pronuclear microinjection involving such a period of time, if coupled with effective blastocyst pre-screening to select for the small number of transgene–containing embryos, might be a feasible means of performing human germline gene therapy. However, reported pronuclear microinjection efficiency values are significantly lower for most mammals other than mice. If human pronuclear microinjection turned out to have a similar efficiency as that obtained with sheep or pigs, then the time taken per genetically modified individual would be ca. 5-fold longer – i.e. more than 2.5 years. And if the rate of transgenesis turned out to be similar to that obtained with cattle, the time would extend beyond 8 years. The efficiency of transgene uptake through pronuclear microinjection is simply not known for humans, nor can it be known a priori. Thus, a circular problem exists: only if the efficiency turned out to be fortuitously high (i.e. similar to murine rates) would there be any point in attempting the technique with humans – but the necessary data on efficiency could only come from actual attempts with humans.

Another problem associated with pronuclear microinjection concerns transgene expression. Only around 60% of pronuclear microinjection-derived mice show transgene expression. Furthermore, in the animals showing expression, there are frequently problems of low-level expression or inappropriate expression (e.g. non-tissue-specific, non-temporal). Accordingly, pronuclear microinjection as a means to human germline gene therapy requires improvements in transgene expression. It is the non-targeted nature of transgene integration associated with pronuclear microinjection that is the root cause of expression problems. Some improvements may come from advances in transgene design, such as the use of matrix attachment regions (MARs) or locus control regions (LCRs): placed on either side of a gene within a transgene construct, these 'insulator' sequences appear to allow the gene to occupy a separate chromosomal domain and thus avoid position-related expression problems (Smith 2002c). However, the best solution would be to target transgenes to precise genomic loci, and at present this is not possible with pronuclear microinjection. Given the fact that even the best designs of targeting transgene undergo random integration more frequently than targeted integration, the only foreseeable way to achieve high

efficiency gene targeting with pronuclear microinjection would be to stimulate homologous recombination (HR) by co-injecting appropriate recombinase enzymes with the transgene. However, elucidation of such enzymes is at an early stage, and it remains to be seem whether this approach could ever provide the quantum leap improvements in targeting efficiency that would be required in the case of human germline gene therapy.

Random integration also raises the concern that an endogenous gene will be damaged by transgene insertion. The degree of risk for any one insertion event must approximate to the proportion of coding sequences (plus controlling elements) within the human genome, a figure of no more than 2%. Thus, endogenous gene damage may be expected to occur in around 1 in every 50 human zygotes integrating transgene DNA. In embryos sustaining such damage, there are several possible outcomes: (a) where a developmentally crucial gene is damaged, the result is likely to be embryo death, and the subsequent non-appearance of a genetically modified individual; (b) where one allele of an important gene is affected, haplosufficiency may permit the development of a normal or near-normal genetically modified individual; (c) where a non-essential gene is affected (such as an allele for hair colour, or a repeated gene), the resulting genetically modified individual may contain a phenotypic change that has no health implications; or (d) where an important gene is affected, debility is likely to occur in the resulting genetically modified individual. Outcomes (a-c), while not desirable, would not necessarily be highly problematic, and the occurrence of these outcomes means that the undesirable outcome (d) would occur at a frequency significantly lower than 1 in 50 genetically modified individuals. Nevertheless, such magnitude of risk implies that pronuclear microinjection in its present stage of development is not acceptable as a means to human germline gene therapy.

4.3.2 Retroviral Transfer

Retroviral transfer remains an alternative to pronuclear microinjection in the context of human germline gene therapy. Traditional RVVs would be of minimal potential

use, due to the high levels of mosaicism associated with these vectors. However, the new generation of lentiviral vectors would avoid such problems (Ikawa et al. 2003; Lois et al. 2002; Pfeifer et al. 2002). These vectors have the additional advantage of high gene transfer rates (70-80% of animals born are transgenic). Accordingly, lentiviral vectors represent plausible candidates for human germline gene therapy. However, the small insert capacity (9-10 kb) would preclude the transfer of many human genes. Additionally, control possibilities are less with RVV delivered transgenes compared with transgenes delivered by microinjection.

The safety problems associated with RVVs (insertional oncogenesis, viral reactivation) would also be a major concern (Cornetta et al. 1991; Gunter et al. 1993; Temin 1990). In principle, judicious genetic alteration of the lentivirus genome would ensure that the resultant vector would have a very high level of safety. However, given the critical context of human germline gene therapy, one would have to question whether our basic scientific understanding of retroviruses is sufficiently advanced to empower rational vector design. Somatic gene therapy provides a salutary lesson here. Human trials involving several hundred patients have been carried out for over a decade using RVVs. Despite the theoretical risks referred to above, a lack of reports of serious adverse affects has resulted in a growing acceptance of the practical safety of RVVs. However, it has been recently reported that two patients (both young children) being treated for X-linked severe combined immunodeficiency disease (SCID-X) using RVV-based vectors have developed leukaemia. In both patients, RVV had integrated into a gene (LMO2) known to cause leukaemia if activated inappropriately. It is not known why the same endogenous gene had been targeted by the RVV concerned. The full cause of leukaemia in these patients is still under investigation, however the fact that both patients share the same integration site, coupled with the fact that the patients were both from the same (10-patient) trial, strongly implicates the particular RVVs employed in this trial (Buckley 2002; Gansbacher et al. 2003; Kaiser 2003). Indeed, clinical trials involving this particular RVV-based therapy have been halted pending further investigations and pre-clinical trials (Fox 2003). It is to be hoped that enhanced RVV design will prevent any recurrence of iatrogenic leukaemia or similar serious adverse affects in somatic gene therapy. However, the occurrence of such adverse RVV effects lends weight to the argument that more basic virology is needed before any potential human germline

gene therapy RVV could be deemed sufficiently safe. At the very least, extensive *in vitro* (cell culture) and *in vivo* (mammalian transgenesis) experimentation would be required in order to establish the safety of any proposed RVV (lentiviral-based or otherwise) for human germline gene therapy.

4.4 Microinjection of Retroviral Vector

As discussed previously (Section 2.1.8), a combination of microinjection with retroviral vectors has been the only approach that has permitted the generation of transgenic primates thus far.

Given the success with primates of microinjection of RVV into oocytes, this approach is likely to be effective for human germline gene therapy. The drawbacks would be similar to those associated with (a) microinjection (i.e. embryo loss) and (b) RVVs (i.e. transgene size limitations, problems with control of expression, safety risks). The process would also be laborious, but it would be expected to avoid the problems of mosaicism associated with most RVVs. Additionally, this form of gene transfer might require fewer eggs than required for pronuclear microinjection. The reported overall rate of transgenesis with rhesus monkeys was 1.3%; although this compares unfavourably with murine efficiencies (up to ca. 6% transgenic), it is significantly better than the rates achieved for animals such as sheep, cows and pigs. Moreover, this 'combined' technique is in its infancy, and its efficiency may well improve with use.

4.5 Sperm-mediated Gene Transfer

The fundamental principles of sperm-mediated gene transfer (SMGT) were discussed in Section 2.1.7. If SMGT does indeed work as reported by some researchers, or if it can be made to work, then this would have very profound implications for human germline gene therapy. Gene transfer into embryos (using pronuclear microinjection or RVVs) is inherently very costly and technically demanding, due in large part to the

need to remove embryos from the female, to manipulate the embryos and finally to return the embryos to the reproductive tract. By contrast, SMGT coupled with AI would permit germline GM with minimum levels of expense and expertise, as would SMGT coupled with testicular injections. Thus, SMGT would in principle permit the widespread use of human germline gene therapy: relatively poor countries would be able to use the technique, and highly centralised facilities would not be required. Of course, such easily available human germline gene therapy would raise serious ethical concerns.

However, even if SMGT were to prove effective as a means to gene transfer, it would be fundamentally limited in the context of human germline gene therapy due to its unsuitability as a means of gene targeting. This limitation is of course shared with the embryo-based gene transfer methods considered above. However, there are at least some glimmers of hope for future gene targeting possibilities in the case of embryo-based approaches: some (albeit very limited) success has been achieved with targeting RVVs (Ellis and Bernstein 1989), and the low natural rate of HR in zygotes might conceivably be increased if appropriate recombinase enzymes were to be discovered and co-injected (Smith 2001). By contrast, there have been no reports of gene targeting using SMGT, and it is difficult to envisage even in outline how this might ever be achieved.

4.6 Episomal Possibilities

As discussed previously, various extrachromosomal plasmid vectors (episomes) have been used as transgenes (Section 2.2.3). In the context of human germline gene therapy, such vectors offer the potential advantage of eliminating the threat to genome integrity associated with uncontrolled genomic integration. However, the previously-mentioned issues of plasmid instability observed in transgenic animals (i.e. fluctuating copy numbers, plasmid loss, abnormal inheritance) represent a major problem. Of similar concern are the health problems (such as tumour formation) that have been associated with some episomal vectors. Of course, the behaviour of an episome must relate in large part to its genetic constitution, and therefore stability problems and

safety limitations may in principle be surmounted by improved plasmid design. However, until such improvements are realised, episomal plasmid vectors could not be considered for human germline gene therapy.

Autonomous artificial mammalian artificial chromosomes (MACs) have been constructed and successfully used to create transgenic animals in which the MACs are maintained autonomously (Section 2.2.3). In the context of human germline gene therapy, these specialised constructs would be expected to give a number of benefits compared with integrated transgenes, including higher and more controllable expression. More speculatively, MACs may be able to function as genetic 'platforms' for the safe subsequent receipt of incoming transgenes. Although this technology is in its infancy, MACs would appear to hold significant future potential for human germline gene therapy (Choo 2001; Co et al. 2000; Grimes et al. 2002; Hadlaczky 2001; Lipps et al. 2003).

4.7 Totipotent Cells

At present, gene targeting requires the use of *in vitro* selection in order to enrich for rare targeted cells amongst a majority of random integration cells. *In vitro* selection cannot be conducted on embryos or sperm cells. Consequently, gene targeting in the context of human germline gene therapy would require gene transfer to be carried out with some form of dividing cells *in vitro*.

4.7.1 Embryonic Stem Cells

As previously discussed, embryonic stem cells (ESCs) can be used to produce gene targeted transgenic mice (Section 3.1.2). In 1998, Thomson et al. isolated ESCs from human blastocysts (Thomson et al. 1998). Subsequently many other researchers have also isolated human ESCs (Amit et al. 2004; Conley et al. 2004; Park et al. 2003; Reubinoff et al. 2000), and a new field in biology has resulted. Furthermore, gene targeting has been achieved in human ESCs (Zwaka and Thomson 2003). However,

totipotency has not been demonstrated in any human ESC line. Unfortunately, this may prove to be a rather intractable situation: proof of totipotency could only (given current technology) come from the establishment of a chimeric human being. It is manifest that the necessary experiments required to pursue this goal would be ethically unacceptable. Thus, the ESC route presently remains firmly closed against human germline gene therapy.

4.7.2 Nuclear Transfer Possibilities

As discussed previously (Section 3.1.3), transgenes can be introduced to somatic donor cells *in vitro,* permitting targeted germline genetic modifications in transgenic animals following nuclear transfer (NT). Accordingly, it seems probable that the technique could in principle be readily applied to humans as a means to achieving germline modifications.

However, the previously mentioned low efficiencies obtained using NT with animal systems would, presuming reconstructed human embryos to behave similarly, represent a potential problem for human NT-based germline gene therapy. Although embryo pre-selection could be used to ensure that only transgene-containing embryos were allowed to gestate, the problem would remain that a large number of valuable donor eggs would be required for each GM attempt.

The poor health status of first generation NT-derived animals (Section 3.1.3) represents a critical problem for human NT-based germline gene therapy. Until such time as the health problems are solved, NT appears to be too dangerous to consider for human germline gene therapy. This is unfortunate, because without NT (or human ESCs) the *in vitro* selection required for germline gene targeting cannot be conducted. Thus, germline GM in humans would be restricted to 'add-in' alterations; gene knockout and gene repair germline alterations in humans are not a practical proposition with the technology available at present.

4.7.3 Non-selective Gene Targeting Possibilities

As discussed in Section 3.1.4, unusually high levels of gene targeting have been reported following the use of (a) isogenic transgenes and (b) adeno-associated virus (AAV) vectors. These reports suggest that in ESCs and in certain somatic cells the efficiency of gene targeting can be sufficient to bypass the need for selection. Selection-based gene targeting places limits on transgene design, due to the need to engineer the requisite selective elements into the transgene. Therefore, the ability to conduct gene targeting in somatic cells without the need for selection would be a welcome addition to the armamentarium of gene transfer technologies that may in future permit human germline gene therapy.

4.8 Conclusions

It is probable that the human germline could be readily manipulated using current transgenic techniques. To achieve this, pronuclear microinjection would probably be effective, as would retroviral transfer, particularly using lentivirus-based vectors. A combination of microinjection and RVVs would probably be most effective – indeed such a combination has recently given rise to the first transgenic primates. SMGT may be effective also, at least in some forms of the approach, such as transgenICSI. However, AI-based SMGT is not yet an established method of transgenesis, therefore the prospects for this potentially very important form of gene transfer are less certain. Totipotent human ESCs have not been established for humans, thus ESC-based gene transfer remains – despite its effectiveness in mice – unavailable for human germline gene therapy. The lack of human ESCs leaves NT-based gene transfer as the only method that might be able to permit gene targeting in human germline gene therapy. NT could probably be readily applied to humans; however, the high level of health problems observed in first generation NT-derived animals render the approach in its present form unfeasible for human germline gene therapy. Table 1 summarises the key features of the major candidate methods that might serve to achieve human germline gene therapy.

If human gene transfer technology is limited to adding-in gene functions via non-targeted transgene integration, and if the process needs be performed on individual embryos isolated from the reproductive tract, it is likely that human germline gene therapy will remain insufficiently safe, excessively inefficient and of inadequate clinical value to permit its use. The widespread availability and applicability of safe and effective human germline gene therapy would require the development of gene transfer methods that would (a) permit gene targeting while (b) avoiding the need for *ex vivo* embryo isolation and manipulation. Unfortunately, at present these two requirements are mutually exclusive. Laborious manipulations involving large numbers of embryos would in principle best be avoided by the use of SMGT, either *in vivo* or *ex vivo*. However, high-efficiency gene targeting is not available at present without the use of *in vitro* selection. Thus, the widespread use of human germline gene therapy does not appear likely to flow from incremental improvements in current GM methods. Rather, widespread human germline gene therapy would appear to be a future possibility that must await substantial scientific advancement. Naturally, it is impossible to predict when such improvements might be forthcoming.

TABLE 1 Selected Gene Transfer Methods

	Pronuclear micro-injection	Retroviral transfer	Micro-injection of Retroviral Vector	Sperm-mediated Gene transfer	Embryonic Stem Cells	Nuclear Transfer
Gene targeting possible?	No (rate is too low)	No (not yet established)	No (not yet established)	No (not possible)	Yes	Yes
Overall efficiency	Up to ca. 6%	Up to ca. 80%	Presently 1.3%	Up to ca. 80%	Up to ca. 25%	Up to ca. 4%
Cost / Expertise require-ments	Very high	High	Very high	Low (except for transgenIC SI and SMGT+IVF)	Very high	Very high
Genetic damage risk	< 2%	High: varies depending upon vector	High: expected to vary depending upon vector	< 2%	Low if gene targeting involved; some epigenetic problems	Low if gene targeting involved, but serious epigenetic problems
Mosaicism (F_0)?	Yes: ca. 65%	Not necessarily (lentiviral vectors)	No	Yes (likely to be similar to pronuclear micro-injection)	Chimeric	No
Transgene capacity	Unlimited: could even be used to deliver MACs	9-10 kb	9-10 kb	Not known	Depends on transfection method	Depends on transfection method
Express-ion	Often low or aberrant, due to random integration	Control possibilities limited by viral sequences	Control possibilities limited by viral sequences	Likely to be low or aberrant, due to random integration	No problems in gene targeted outcomes	No problems in gene targeted outcomes
Scientific status	Fully established in non-primate animals	Well established in non-primate animals	Early success reported in primates	Not well established despite several reports of success – theoretical difficulties	Fully established – but only in mice	Becoming well established

TABLE 1 (continued) Selected Gene Transfer Methods

	Pronuclear micro-injection	Retroviral transfer	Micro-injection of Retroviral Vector	Sperm-mediated Gene transfer	Embryonic Stem Cells	Nuclear Transfer
Use in human germline GM condition-al upon scientific break-throughs?	No	No	No	No	Yes: human ESCs required	No
Scientific advances required before method could become a practical proposit-ion for human germline gene therapy	Incremental improvemen ts in efficiency ICM pre-screening technology Use of 'insulator' sequences in transgenes	RVV design improved and tested to ensure safety Engineering of RVV genome for improved transgene expression	RVV design improved and tested to ensure safety Engineering of RVV genome for improved transgene expression	Establish-ment of SMGT (must be reliable and repeatable) Developme nt of augmented uptake methods	n/a	Improve-ments in reprog-ramming to avoid epigenetic problems Incremental improve-ments in efficiency
Ideal improve-ments	Gene targeting via recombinas e use	Gene targeting by engineering RVV genome	Gene targeting by engineering RVV genome	Establishme nt of AI-based SMGT as a reliable form of GM	n/a	n/a

Chapter 5: Potential Applications for Human Germline Gene Therapy

5.1 Introduction

For any human germline therapy application to be sanctioned, it ought to offer considerable benefits to humans. In respect of therapeutic benefits for living patients, germline approaches could of course offer no hope. Rather, only (unborn) offspring could hope to benefit. For such individuals, hereditary genetic diseases would be the main candidates for treatment: disorders such as infectious diseases or most cancers affect people in largely unpredictable, non-genetic ways.

5.2 Germline vs. Somatic Approaches

Two distinct approaches are possible when attempting to genetically alter somatic cells. One approach involves the removal of cells from the patient's body; the cells are then genetically manipulated and subsequently returned to the patient's body. The other approach involves the manipulation of cells where they reside naturally in the patient's body. These two approaches are termed *ex vivo* and *in vivo* therapies, respectively. For both *ex vivo* or *in vivo* approaches, and regardless of the cell type involved, the central feature of all somatic gene therapies is the need to successfully deliver therapeutic genetic material to the target cells.

Both *ex vivo* and *in vivo* somatic gene therapies are under investigation and have been attempted in respect of a wide range of conditions. To date only a handful of patients have shown clear clinical improvements attributable to gene therapy. However, some promising results have recently been obtained.

Severe combined immunodeficiency disease (SCID) provides a prime example of successful somatic gene therapy. Children who inherit two defective alleles for adenosine deaminase (ADA) develop this disorder. Similarly, male children who inherit a single defective copy of an X-linked interleukin receptor subunit (γC) gene develop severe combined immunodeficiency disease (SCID-X). Several clinical trials of gene therapy for both ADA-SCID and SCID-X have been conducted, using *ex vivo*

approaches. The general approach has been to infect peripheral blood lymphocytes or bone marrow stem cells *in vitro* with retroviruses engineered to contain the required gene (ADA or γC), the genetically manipulated cells then being transplanted to patients. Results from the ADA-SCID trials demonstrate that some of the treated children produce ADA, with the clinical condition of these children being markedly improved. Some caution is required when interpreting the clinical improvements because some of the patients also received ADA protein as a treatment. Results from recent SCID-X trials have been especially promising: T-cell, B-cell and NK cell counts have been restored in several patients, to the extent that the disorder has apparently been complete cured (Aiuti et al. 2002; Cavazzana-Calvo et al. 2000; Cavazzana-Calvo et al. 2001; Hoogerbrugge et al. 1998; Otsu and Candotti 2002). However, it is salutary to note that two patients have developed leukaemia following RVV-based gene therapy for SCID-X.

Many other recessive single gene disorders are currently under intensive investigation for gene therapy. Promising preliminary results have been obtained in respect of several disorders including Duchenne muscular dystrophy (Chamberlain 2002), emphysema (Stecenko and Brigham 2003), phenylketonuria (Eisensmith et al. 1999), retinitis pigmentosa (Dejneka and Bennett 2001), and sickle cell anaemia (Pawliuk et al. 2001). Although clinical success to date has been limited, gene therapy is certainly in its infancy: a lack of spectacular results at so early a stage should not be taken to indicate that gene therapy will not become highly efficacious given time. Single gene recessive disorders remain good candidates for the development of gene therapy strategies and, given that a great deal of preliminary work has already been carried out with this class of disorders, it seems reasonable to expect progress in this area before others. Nevertheless, somatic gene therapy has also been proposed for dominant and polygenic disorders.

Somatic gene therapy also holds promise for non-inherited conditions, such as cancer. A multitude of *in vivo* and *ex vivo* anticancer therapies are currently under development (Heo 2002; McCormick 2001; Wadhwa et al. 2002). Indeed, the majority of all human gene trials to date have been aimed at the treatment of cancer. The field is rapidly advancing and eclectic, and progress is likely to accelerate as

basic knowledge of cancer biology increases. Other disease categories that may in principle be treatable by somatic gene therapy include infectious diseases (Bunnell and Morgan 1998; Mautino and Morgan 2002; Statham and Morgan 1999), autoimmune disorders (Fathman 2002; Tarner and Fathman 2002), and even trauma (injury) (Blits et al. 2002; Palmer et al. 2002; Yang et al. 1997). Indeed it is difficult to name categories of disease that could not in principle be seen as potentially treatable by somatic gene therapy, such is its potential power.

Thus, although in its infancy, somatic gene therapy holds great promise for treating disease. Given its potential power, it is tempting to take the view that somatic gene therapy renders its germline equivalent superfluous. However, germline gene therapy has several intrinsic advantages over its somatic counterpart.

A major potential advantage of germline therapy is the permanent nature of the genetic alteration. In many disorders, transgene-containing cells would gradually be replaced by non-transgenic cells. This problem would apply to all disorders except those involving quiescent cells – some CNS disorders might fit into this category. This problem could only be avoided if stem cells for the affected tissues were genetically altered. This is problematic for *in vivo* approaches because, as a rule, stem cells tend to reside deeply within tissues and are therefore less accessible to transgene vectors in comparison with their (functional) daughter cells. *Ex vivo* somatic gene therapy may be used in some cases, such as haematological disorders in which pluripotent clonogenic stem cells may be subject to *ex vivo* manipulation for subsequent return to the body to recolonise the bone marrow. However, it would be implausible to suppose that all types of stem cells could be treated in this way. Moreover, many of the *in vivo* gene transfer methods applicable for somatic gene therapy result in a non-permanent presence of the transgene in most or all of the cells treated. Such methods include most viral vectors (with the notable exception of RVVs), liposomes and electroporation. Of course, repeated application of a somatic gene therapy may not be a major problem. For example, in the case of lung disorders such as cystic fibrosis (CF) or emphysema, intensive research is underway to develop gene delivery systems based on inhaling viral vectors or liposomes. If such systems prove effective, then it is unproblematic to countenance repeated use of inhalation-based somatic gene therapy by individual patients, considering that inhalers are

intrinsically non-invasive and are in common use to deliver conventional drugs. However, less accessible organs or tissues would require more invasive procedures for re-delivery of transgenes. Germline gene therapy should avoid all problems of non-permanence, regardless of the origin of the problem (i.e. transgene loss through cell replacement or transient transfection).

As indicated previously, *ex vivo* GM appears to be the most promising somatic gene therapy approach, given presently available gene delivery technology. However, many cells in the body are non-removable, or are non-dividing (or at least non-clonogenic). *Ex vivo* approaches are not applicable to diseases involving such cells. Thus, for several disorders potentially amenable to somatic gene therapy, gene transfer would have to be of the less effective *in vivo* variety. Indeed the range of disorders not suitable for *ex vivo* gene transfer is very broad, and includes disorders of the muscular system (e.g. Duchenne muscular dystrophy (DMD)), the respiratory system (e.g. CF) and the brain (e.g. Lesch-Nyhan syndrome). In such cases, except where *in vivo* somatic gene therapy (and conventional medical approaches) proves effective, a germline gene therapy might be the best or only option.

In many disorders, more than one part of the body is affected. For example, in DMD the entire skeletal muscular system is affected. Moreover, in some disorders disparate tissue or organ types are affected. For example, in CF the gastrointestinal tract (GIT) is affected (often severely) in addition to the respiratory system. Thus, curing such disorders by somatic gene therapy would require transgenes to be delivered to a large number of sites in the body, with such sites often comprising different cell types. This presents formidable problems in terms of invasiveness and vector design. By contrast, germline gene therapy has the potential to place transgenes in cells throughout the body, and by only one gene transfer operation. Thus, germline gene therapy may be the best or only option for disorders in which many body sites require treatment.

Many of the serious genetic conditions potentially amenable to somatic gene therapy often kill or seriously damage patients in their first months or years of life. Indeed, as a generalisation, conceptually there exists an inverse correlation between the age of the patient and the probable effectiveness of gene therapy. Accordingly, the foetus is often viewed as an ideal developmental stage for somatic gene therapy. In addition to

permitting treatment prior to irreversible disorder-induced damage, foetal somatic gene therapy has a number of potential advantages compared with later-stage approaches (Coutelle and Rodeck 2002; Kaplitt et al. 1998). These advantages include: (a) better transgene uptake efficiencies due to the high percentage of rapidly dividing cells populating the foetus (as discussed previously, some gene transfer approaches do not function well with non-dividing cells); (b) the possibility of generating whole-tissue/organ alterations by genetically altering a small number of appropriate clonogenic cells (such cell-types are a feature of early foetal development); and (c) the avoidance of an immune response against transgene vectors due to the preimmune status of the early foetus. Thus, given the scientific advantages associated with the foetus, somatic gene therapy may well come to be frequently targeted at the unborn child. In foetal gene therapy there can obviously be no consent from the 'patient' affected (beneficially or detrimentally) from the treatment. However, foetal gene therapy is far less developed than other forms of somatic gene therapy. Thus, the efficacy of foetal stage intervention remains to be established. Worse, given the sensitive stage of development involved, the risk of iatrogenic damage (possibly including an enhanced risk of inadvertently transfecting germline tissues) is intrinsically greater than the risk attached to treating children or adults. Consequently, germline gene therapy may be preferable over foetal somatic gene therapy as a means of early intervention in disorders involving developmental damage.

If both somatic and germline approaches were equally able to cure (or avoid a patient developing) a given disease, then the latter approach may be preferable, because a germline correction would prevent inheritance of the hitherto damaged or missing gene. Thus, the offspring of the germline gene therapy 'patient' would have a greatly reduced risk of developing the disorder. (Following germline gene therapy, the resultant 'patient' would either be (a) free from the disease gene or (b) have the carrier genotype, in the case of recessive disorders dealt with by simple gene-addition.) Thus, germline gene therapy has the marked advantage over somatic gene therapy of offering a permanent elimination of disease-causing genes. Looking wider than the benefits that germline gene therapy (assuming it to be safe and efficacious) would bring to the line of descendants from the treated 'patient', germline gene therapy would have a positive effect on the human gene pool. Clearly, the magnitude

of this effect would be a function of (a) the extent to which human germline gene therapy is employed within the human population and (b) the frequency of each disease-causing gene in the population. Germline interventions involving only a few families for a very rare genetic disorder would have minimal impact on the gene pool, whereas widespread human germline gene therapy against a common disease (CF for example) would be expected to have a greater effect on the gene pool. Regardless of the magnitude of effect, each instance of successful germline gene therapy against any serious genetic disorder would contribute a measure of improvement in the human gene pool.

5.3 Human Germline Gene Therapy vs. Pre-screening

In cases where two heterozygotes wish to have children, medical science already provides methods for pre-screening embryos or foetuses for genetic defects, such that the parents of homozygous foetuses can decide whether the child should be born. Similar considerations would apply to X-linked disorders, with the additional possibility of sex selection. Polygenic disorders should also be amenable to pre-screening. For dominantly conferred disorders, pre-screening of embryos would again be the logical route. In cases where individuals were homozygous for dominantly conferred disorders, germline gene therapy might be the only option, because 100% of offspring would include a disease-causing allele. However, very few if any autosomal dominant disorders have been described where the homozygote is alive and in a fit enough state to reproduce while the heterozygous combination yields a serious medical condition.

Indeed, there is in effect a 'golden rule' applying to disorders potentially amenable to germline gene therapy: in any disorder with enough molecular knowledge available to allow the prospect of germline gene therapy, that same knowledge will also be sufficient to allow detection of the disease-causing sequences via genetic pre-screening.

Accordingly, as far as individual parents are concerned, genetic pre-screening offers a more viable alternative to human germline gene therapy. Of course, in principle this could change if germline manipulation was to benefit from a 'quantum-leap' improvement in terms of its precision and efficiency, arising from dramatic future technological improvements. If such a speculative advance was to occur, it could in principle make germline gene therapy easier than, and therefore preferable to, pre-screening. However, with present gene transfer efficiency levels, pre-screening would clearly be a better choice than human germline gene therapy. It is conceivable that 'pro-life' parents, implacably opposed to abortion or embryo discard might prefer human germline gene therapy to pre-selection (although such parents would have to embrace a doctrine of double-effect in order to accept the 'accidental' loss of embryos likely to be entailed by human germline gene therapy). Nevertheless, it seems reasonable to presume that a majority of parents would simply choose the most effective means of ensuring that their offspring are free from disease. Consequently, given present gene transfer efficiencies, pre-selection would seem to take precedence over human germline gene therapy.

5.4 Candidate Conditions for Human Germline Gene Therapy?

Notwithstanding the above-mentioned advantages of pre-screening over human germline gene therapy, it is possible to identify a number of important contexts in which germline gene therapy may plausibly offer a potential role to individual parents. One attractive possibility concerns infertility. In some genetic forms of male infertility, specific mutations cause immotility of sperm cells. In men with such infertility, SMGT might be used to deliver corrective (simple add-in of function) transgenes to spermatogonia *in vivo*. Assuming the levels of success reported with mice (Section 2.1.7), more than 50% of sperm may pick up transgene DNA; such high efficiencies would produce adequate numbers of sperm for IVF. Amongst ejaculated sperm, it should only be the successfully modified sperm that should be motile. Thus, transgenic sperm would be able to fertilise an egg, but non-transgenic sperm and sperm in which the transgene was present but not expressing would remain immotile

and hence unable to participate in fertilisation. At present, genetic forms of male infertility involving sperm immotility can be successfully treated conventionally using ICSI. However, the disadvantage of ICSI is that the aberrant (infertility) gene stands a high probability of being passed on to the next generation. By contrast, human germline gene therapy by *in vivo* SMGT offers the potential advantage of transmitting the transgene to the next generation, thus providing a permanent solution that would benefit subsequent descendants of the original patient.

Another important context for the potential use of human germline gene therapy is where several deleterious genes are being considered simultaneously in one (potential) individual. Cutting edge genetic advances such as the human genome project are facilitating the elucidation of a growing array of deleterious genes, many of which probably operate as risk factors, and it is likely that many or even most humans will each have in their genome a number of these deleterious genes. To take a hypothetical example, consider a pair of prospective parents: the genome of one parent contains a gene that acts as a strong risk factor for the development of senile dementia, while the other parent's genome contains (a) a gene that acts as a risk factor for autism and (b) an oncogene predisposing that individual to a certain form of cancer. Clearly, it would be desirable to avoid any of these three deleterious genes in the offspring from these parents. In principle, pre-screening early embryos could achieve this. However, as soon as more than one gene needs to be avoided, the number of embryos required for pre-screening increases. In the present example, on average only one in eight embryos would be free from all three deleterious genes. Thus, in cases in which it is desirable to deal with several deleterious genes at once, pre-screening becomes inherently less effective, simply due to the limited numbers of human embryos available (from each woman) for analysis. In such cases, germline gene therapy might be the best - or only - tool available.

5.5 Genetic Enhancement

In contrast to germline gene *therapy,* in which the goal is to avoid or mitigate an otherwise inevitable disorder, genetic *enhancement* implies the manipulation of general phenotypic traits (where such traits are under genetic control, and hence potentially amenable to GM) to produce 'improved' individuals. Such phenotypic traits might include natural life span, height, disease resistance, IQ, body-shape, size of facial features, etc. One commonly expressed fear concerning human germline gene therapy is that the techniques of GM necessary for human germline gene therapy might be used for such non-therapeutic reasons.

Assuming that germline GM techniques become effective in dealing with human disease, it is undoubtedly true that the same techniques could be applied for enhancement purposes. Thus, the issue becomes an ethical one, involving several questions including: (a) should germline enhancement be permitted? (b) if germline enhancement ought to be totally proscribed, should therapeutic forms of germline GM also be banned lest on the grounds that human germline gene therapy would function as a 'slippery slope' towards germline enhancement? And (c) if *some* germline enhancement is permissible, where should the limits be set? Such ethical questions are addressed later in this thesis.

At this point, it should be recognised that no clear boundary necessarily exists between therapeutic GM and enhancement GM. For example, germline GM used against Alzheimer's disease might also be effective in enhancing memory *per se,* in people not predisposed to the disorder. Similarly, germline GM used against a particular inherited cancer might also provide a general resistance against cancer in non-susceptible people. Another example would be germline GM to treat musculo-skeletal disorders, where similar GM applied non-clinically might enhance body-shape or even athletic potential. Although these examples are necessarily speculative and futuristic, they serve to demonstrate that germline GM cannot be simply divided into therapeutic versus enhancement categories.

5.6 Gene Pool Improvement

A distinction can be made between the use of germline GM techniques for (a) individual parent/child benefits and (b) improvement of the human gene pool. This distinction cuts across the therapy vs. enhancement issue, because the genetic changes involved at both parent/child and gene pool levels could be for either therapeutic or enhancement purposes.

On first sight, the notion of applying germline GM to the entire human population would appear impracticable. However, similarly ambitious public health programs have been proposed or employed in conventional medicine. The clearest examples come from attempts to prevent serious infectious diseases: vaccination programs have been aimed, with great success, against childhood diseases such as smallpox and polio.

Given safe, efficacious and low-cost germline gene transfer technology, future global health programs might in principle be used to improve the human gene pool. Certainly, it would be erroneous to assume that the human genome *per se* is not potentially amenable to improvement. For example, it is now well accepted that evolutionary pressures have been relatively weak in the context of late-onset (post-reproductive age) disorders such as cancer, the result being that humans possess only a modest degree of cancer resistance. Germline GM technology could in principle be used to add 'anti-cancer' genetic sequences (such as extra checkpoint genes or tumour-suppressor genes) into the human genome. Assuming that these additional anti-cancer genes would greatly reduce the risk of cancer, this form of germline modification, carried out as widely as possible, would ensure that future generations of people inherited enhanced levels of protection against cancer.

Chapter 6: Bioethical Framework

6.1 Preface

At the outset, it is important to note that this chapter (and the next) differs fundamentally from the foregoing chapters. While Chapters 2-5 are concerned with *scientific* issues related to human germline gene therapy, the Chapters 6 & 7 of this thesis deal with *ethical* issues. This is most obvious in terms of reference citations. Due to a custom/practice mismatch between ethics and science, ethics articles tend to contain far fewer reference citations. This mismatch is logical, in that few of the basic ideas employed in ethics discourse can be clearly attributed to one particular author/original article. This is in contrast to scientific findings & theories, which are more distinct and thus have clearer-cut origins in the literature. Moreover, some of the ideas are so well known (by ethicists) that reference citation is rendered largely redundant. This is analogous to there being no need for science to cite references for well-known matters, e.g. "viruses are infective agents smaller than common microorganisms, requiring living cells for multiplication". Furthermore, in this thesis, many of the ideas and examples in the following chapters are in fact original ideas (or originally synthesised ideas) produced by the present author.

Accordingly, the reader can expect to find far fewer reference citations in Chapters 6 and 7 in comparison with the previous chapters. Where citations do occur, these refer either to (a) original, seminal works introducing or developing major concepts; (b) recent works that place core concepts in a relevant context; (c) publications that articulate a specific point or example; or (d) general works that provide useful background. Note that all the examples (cases) given are original, except where specifically referenced. This minimal use of references is consistent with the usage in my papers that deal with bioethical issues submitted as part of the portfolio of published work accompanying this thesis: *Animal Genetic Manipulation: A Utilitarian Response* (Smith 2002c); *Gene Therapy: Theoretical and Bioethical Concepts* (Smith 2003b); *Human Germline Genetic Modification: A Utilitarian Bioethical Perspective* (Smith 2005).

A second fundamental difference between Chapters 2-5 and Chapters 6 & 7 is that first-person language is commonplace in that latter, but avoided in the former. This differential use of first-person language is deliberate. Scientific publications are customarily written in impersonal style. This reflects the impersonal nature of the data, findings and theories with which science is concerned. By contrast, ethics journal articles, including medical/bioethics journal papers, are commonly written using first person language. For example, this personal style is the norm in articles in the journal *Bioethics,* including the article in that journal submitted as part of the portfolio of published work for this thesis (Smith 2002b). Such use of first person language in ethical discourse is useful, because it allows readers to observe an author constructing his/her ethical arguments. In contrast to the data/fact-driven development of scientific arguments, ethical arguments inevitably require a degree of subjectivity.

Accordingly, the custom of using personal language in ethical discourse has been maintained in Chapters 6 & 7 of this thesis.

6.2 Ethics

Although it would be legitimate and interesting to compare in detail a broad range of competing ethical approaches, such an endeavour would be beyond the scope of the present discussion. Moreover, even the most thorough, extensive evaluation of competing ethical approaches would be insufficient to demonstrate the unequivocal superiority of one particular system. Accordingly, the following discussion (Sections 6.3 & 6.4) is limited to (a) providing an outline of the key features of major competing ethical approaches and (b) describing and evaluating the core principles of the ethical approach employed by the present thesis – utilitarianism.

As previously stated (Section 1.6), this thesis does not claim to establish utilitarianism as being the only valid – or even necessarily the best – ethical approach. Instead, I employ utilitarianism as a *tool* for the exploration and evaluation of key bioethical issues arising from the future prospect of human germline gene therapy. Thus, although the bioethical conclusions reached in this thesis (see Chapter 7) are designed

to be consistent with utilitarianism, those who reject the underlying principles of utilitarianism may legitimately reject these conclusions.

Nevertheless, I am inclined to hold a utilitarian position. I suggest that the utilitarian position is a minimal one, an initial point reached by searching for core principles that could in principle be adopted by any rational person or legislative body. To be persuaded to go beyond utilitarianism and accept non-utilitarian principles (such as the sanctity of life, unalienable individual rights, purity, etc) would require strong reasons for doing so. I suggest that non-utilitarian moral principles have not been established as unequivocally valid. Accordingly, I suggest that there are valid grounds for adopting utilitarianism, at least provisionally, as an approximate guide to decision-making.

6.3 The Diversity of Ethical Approaches

Ethical considerations of any problem or issue may usefully be divided into (a) non-consequentialist and (b) consequentialist approaches. Non-consequentialism considers that the action (or even just the motivation behind an action) is the crucial ethical consideration. In other words, the action itself is more important than the actual outcome (consequence) of the action. By contrast, consequentialist approaches hold that outcomes (as opposed to actions) should be the crucial determinants of ethical decisions.

6.4 Non-consequentialism

The major forms of non-consequentialism may be categorised as follows:

- Intuitive Responses
- Religious Laws
- Rights

The following sections consider each of these categories.

6.4.1 Intuitive Responses

An ethics based upon intuitive responses places paramount importance on people's "instincts" and/or "what feels right (deep down)" (Atkinson 1990; Kekes 1986; Levy 2003; Miller 2004; Portmore 1998; Sencerz 1986; Wilson 1996). For example, some individuals would (a) claim to have a strong instinct against animal transgenesis, and thus (b) to hold that animal transgenesis is ethically wrong. In ethical decision-making, intuitive responses have some advantages: they are 'natural' and easy – there may be no need for rational discourse in deciding which actions to take. However, there are several problems associated with intuitive responses in ethics. Such problems include (a) the risk that apparently 'natural' instincts might have been unduly influenced by (say) social, political or cultural pressures; (b) the difficulties that arise when one person's instincts clash with another person's instincts; and (c) the reliance for ethical guidance upon a mysterious 'black box' mental process.

6.4.2 Religious Laws

Religious laws hold that God is the origin of all ethical rules, therefore religious scriptures or rules must form the basis of ethical decision making (Cahill 2003; Green

1997; Messikomer et al. 2001; Rasmussen 2000; Turner 2003). Accordingly, ethical direction arising from religious laws is of an absolutist nature. For example, if a particular religious law stated "do not 'play God' with nature", this would deem all attempts at genetic modification 'wrong', even if it the resultant transgenic organisms would produce a pharmaceutical product of great benefit to people suffering from a serious disease. Religious laws have the advantage of being (at least in some cases) clear and unambiguous. However, there are major (perhaps insurmountable) problems with the use of religious laws as the basis for ethical decision-making. One such problem is the serious lack of agreement that exists between different religions on ethical questions. Related to this, the particular God or Gods from which ethical rules are supposed to have originated will vary depending on the religion of each moral agent. More fundamentally, religious laws are inescapably founded upon a highly questionable basis, namely the belief in the existence of a supernatural God(s). And, even for those who are believers, there remains a major problem: *why* should God's rules be seen as *ethically correct* rules?

6.4.3 Rights

The language of 'rights' is in widespread usage. In the ethical context, a 'right' is something (an 'ethical entity') that an individual (or group) possesses; it is this ethical entity that is to be respected (d'Oronzio 2001; Knowles 2001; Rawls 1971; Thomasma 2001; Thomasma and Loewy 1997). For example, one person may wish to prevent the development of genetically modified pigs because that person believes that the pigs have a 'right' not to be used in this way. At this point, the use of the term 'rights' must be distinguished from its use in non-ethical contexts. In non-ethical contexts, rights are not intrinsic properties of individuals (or groups); rather rights are *conferred* (by society or government, etc). For example, it may be that in a particular country the creation of genetically modified pigs is prohibited by law; in this case, one would be entitled to say that the pigs have a *legal* right (as opposed to an ethical right) not to be subjected to genetic modification (however, it is worth noting that legal rights are often based on ethical considerations).

Ethical rights are usually clear and unambiguous. However, there are serious problems with the whole notion of ethical rights. Disagreement between different people over rights is a major issue. For example, one person may think that destroying bovine embryos of an undesired sex (e.g. male embryos in dairy agriculture) acceptable on the grounds that the embryos do *not* have a right to life, but another person may believe that the destruction is wrong because this person believes that the embryos *do* have a right to life. It is difficult to see how such positions can be reconciled, without recourse to ethical principles beyond 'rights'. And if additional ethical principles are required, then the fundamental validity of ethical rights is called into question – why invoke rights at all, if other ethical considerations are needed? Furthermore, rights can conflict with each other, often with no obvious resolution possible. For example, while accepting that bovine embryos have a right to life, high-tech farmers may at the same time claim that *they* have the right to deal with the embryos – i.e. as part of a right to commercial freedom. Thus it appears that any acceptable (ethical) 'right' would itself have to be based on sound ethical principles. But this requirement suggests that (ethical) 'rights' are not independent entities intrinsically held by individuals (or groups).

6.5 Consequentialism

Consequentialism holds that outcomes (as opposed to actions) should be the crucial determinants of ethical decisions. Two main forms may be discerned:

- 'Non-sentience based' consequentialism
- 'Sentience-based' consequentialism

The following sections consider each of these categories.

6.5.1 'Non-sentience based' consequentialism

Possible desirable non-sentience outcomes include economic consequences (e.g. high gross domestic product (GDP), energy availability per capita, availability of consumer goods) and aesthetic consequences (e.g. beauty in the natural world, highly cultured citizens, artistic artefacts per capita). 'Non-Sentience Based' consequentialism is rarely stated as an explicit moral system. Rather, it is exists as an implicit assumption in several areas of human activity, including economics, art and environmentalism. A major problem with 'non-Sentience Based' consequentialism is that it is difficult to find agreement over which consequences are the most desirable ones. Values such as beauty, culture and art are highly subjective: it is inevitable the importance of such values will vary greatly between different people, depending on many factors such as age, nationality, social group, sex, etc. Economic values may be more consensible than their aesthetic counterparts (although there nevertheless exists a great deal of subjectivity – for example, should a plentiful supply of cheap consumer goods be favoured over an unpolluted urban environment?) However, even highly consensible economic values require further philosophical justification. The question is: Why should an economic value be ethically desirable? For example, it is well accepted by most people that a high GDP is desirable. But the question then needs to be asked: Why should we value a high GDP? There are of course many plausible answers to this question. For example, a high GDP may allow a better healthcare system. It may permit more enjoyable use of leisure time. It may allow better nutrition for more people. The point here is that non-sentient consequences (such as high GDP) are not goods *of themselves.* Rather, the ethical basis of each non-sentient consequence depends on further ethical principles, such as (to continue with the example of GDP) the desirability of good healthcare, nutrition and leisure opportunities for a country's populace. Yet this once again begs the question, because the desirability of good healthcare, nutrition, leisure themselves require (at least at a philosophical level) further justification. Why is good healthcare desirable? Why is good nutrition desirable? To answer such a series of recursive questions, fundamental ethical principles are required. Accordingly, it is difficult to avoid the conclusion that non-sentient consequentialism – although commonplace – is intrinsically weak as an

ethical approach because it is not underpinned *per se* by fundamental ethical principles.

6.5.2 'Sentience-Based' Consequentialism: Utilitarianism

Sentience is the ability to experience sensations. Sentience is possessed by humans and, to varying degrees, by many non-human animals. 'Sentience-based' consequentialism, or utilitarianism, is based on the concept that all values derive ultimately from sentience. Sensations may be grouped into two categories, happiness and misery. In utilitarianism, ethically correct decisions are those that maximise the overall amount of happiness, or reduce the overall amount of suffering (Hare 1963; Mill 1871; Quinton 1973; Sidgwick 1907; Smart 1973). For example, a utilitarian may view it as wrong to produce a particular strain of cosmetic-producing transgenic animals because of the animal suffering entailed by the process of transgenesis. On the other hand, the same utilitarian may consider it right to produce a particular strain of pharmaceutical-producing transgenic animals because it is likely that the animal suffering would be outweighed by the reduction in suffering obtained from the pharmaceutical product (note that utilitarianism does not suggest that 'animal' sentience is inherently less important than 'human' sentience; in fact, utilitarianism must reject this view as 'speciesist'). Regardless of one's cultural and socio-political background, it is difficult to deny that happiness is highly desirable, and misery extremely undesirable. In utilitarianism, the paramount importance of sentience is taken as axiomatic. Accordingly, utilitarianism regards sentience as an 'ultimate principle', because people do not need to provide further reasons for believing that happiness is good and suffering bad. Flowing from this, utilitarianism proponents may be optimistic that (in principle) ethical agreement is possible between all people. Indeed, utilitarianism may be used in order to generate 'rights' that we could (in principle) all agree upon.

6.5.2.1 Key Features of Utilitarianism

Utilitarianism, like all ethical systems, exists in various forms. A taxonomy of the subspecies of utilitarianism lies outside the scope of the present discussion. However, it is useful to consider certain core ideas that are common to all or most forms of utilitarianism:

- Actions are judged in terms of happiness/misery
- Overall sentience must be considered
- Sentience implies respect for equality of interests
- Possibilities must be compared
- Probabilities must be considered
- 'Side effects' must be considered

These key features are discussed in the following sections.

6.5.2.1.1 Actions are judged in terms of happiness/misery

This idea is the most fundamental feature of utilitarianism: as described previously, a course of action is ethically laudable where its consequence is an increase in happiness, or at least a decrease in unhappiness (Hare 1963; Mill 1871; Quinton 1973; Sidgwick 1907; Smart 1973). To take a simple example, it would be ethically correct (and the converse ethically monstrous) for an anaesthetic to be administered to a patient about to undergo major abdominal surgery.

Some forms of utilitarianism view happiness as something that is correlative to desire or want. Accordingly, such forms of utilitarianism consider the maximal satisfaction of human preferences as the central concern of utilitarian theory. However, preference-satisfaction functions as a route (albeit an indirect route) to maximising happiness / reducing suffering (Brandt 1979; Harsanyi 1977; Scanlon 1993). Such desire-fulfilment variants of utilitarianism are probably most useful in the context of

personal ethics and political philosophy. Desire-fulfilment is less useful for bioethical questions of the sort considered in this thesis, where direct effects on sentience are generally more relevant.

6.5.2.1.2 Overall sentience must be considered

This idea avoids the use of the foregoing core idea in an excessively narrow fashion. In many situations it cannot be good enough to consider the sentience of just one person or of one particular group of people (Scarre 1996; Singer 1993). For example, a battlefield surgeon would be wrong to administer scarce analgesic to a lightly injured patient experiencing mild pain if this would deny adequate pain relief to a badly wounded patient enduring terrible pain. It would not be good enough to claim that the improvement in overall happiness in the first patient justifies analgesia for that patient because, if the overall sentience change were considered, the best outcome would be obtained by reserving the analgesic for the second patient (the first patient would be mildly miserable, but the decrease in suffering experienced by the second patient would outweigh the first patient's unhappiness).

6.5.2.1.3 Sentience implies respect for equality of interests

Respect for equality of interests does not imply a political defence of egalitarianism. Rather, the notion of equal interests in utilitarianism arises logically from the central basis of utilitarianism, namely sentience. If sentience is the overriding moral principle, it follows that other, non-sentient characteristics of the individuals in question are not ethically relevant. Thus, non-sentient features such as age, sex, social position, educational background, personality or IQ do not of themselves carry any weight in utilitarian deliberations (Harsanyi 1991; Langford 1992; Stein 2002). For example, the suffering of an illiterate octogenarian is no less serious than the same degree of suffering experienced by a 40-year old high court judge. Similarly, the battlefield surgeon in the previous example would be wrong to allow a patient's military rank to influence the outcome of deliberations on the allocation of scarce analgesia.

6.5.2.1.4 Possibilities must be compared

Utilitarians should not simply accept the first course of action that alters the balance of sentience in a positive direction. Instead, the utilitarian should investigate and compare alternative courses of action, such that the most happiness-enhancing option may be chosen (Glover 1977; Singer 1993). For example, suppose an uncomfortable and distressing medical paediatric procedure would definitely increase happiness by improving the long-term health of the child; however, the gastric procedure would not be ethically justified if the same medical ends could be met by a more humane (e.g. non-invasive) procedure.

6.5.2.1.5 Probabilities must be considered

The need to consider probabilities incorporates simple probability theory into utilitarianism. The less probable a desirable outcome is, the less weight should be attached to the proposed course of action designed to deliver that outcome (Beauchamp and Childress 2001; Hare 1993; Kuhse and Singer 1999; Scarre 1996). For example, a surgical intervention may be proposed to separate a pair of conjoined twins. The separation of the twins would be a desirable outcome, because the twins would be happier if able to lead independent lives separate from each other. On this basis, the surgery ought to be permitted. However, suppose that expert opinion placed the probability of success at approximately 35%, with failure inevitably resulting in the death of at least one of the twins. Given this low probability of success, the ethical case for proceeding with the surgery is debatable. And if the expert opinion had indicated a much lower chance of success, say 5%, then the case for surgery would be concomitantly weaker still. In many cases, the balancing of desired outcome with the probability of success will be an inherently subjective matter: in the conjoined twins example, it may be best to fully inform the patients of the risks and then allow the patients to decide whether to proceed. However, simple calculations are possible and useful in some circumstances. For example, suppose that the following statistics on two (hypothetical) drugs are available (and reliable): drug A saves the lives - and

hence permits an average of 30 further years of happiness per patient - of 70% of patients who would otherwise have died within an average of 5 years; suppose also that the drug leads to the death within an average of 1 year of 20% of patients through adverse reactions. Drug B, by contrast, saves only 50% of patients (giving an average of 30 further years of happiness per patient); but suppose also that this drug is much safer than drug A, accidentally killing (within 1 year) only 2% of patients through adverse reactions. From these statistics, it is possible to compute the average increase in life associated with each drug, as follows:

Drug A: [30yr. x 70%] + [1yr. x 20%] + [5yr. x 10%] = 21.7yr.
Drug B: [30yr. x 50%] + [1yr. x 02%] + [5yr. x 48%] = 17.4yr.

Accordingly, it would be ethically correct to prescribe drug A rather than drug B because, taking the relevant probabilities into account, drug A will deliver a higher average quantity of happiness (i.e. 4.3 extra years of life lived per patient).

6.5.2.1.6 'Side effects' must be considered

Utilitarian deliberations must take into account the 'side-effects' that a proposed course of action may cause (Glover 1977). For example, some ethicists have claimed that infanticide ought to be permissible for infants born with devastating medical disorders, where such disorders would cause a life not worth living (due to a negative balance of sentience). Utilitarian considerations give *prima facie* support to this claim: elimination of a life in which pain and misery outweigh pleasure would give a positive contribution to the overall balance of sentience. However, the probable side-effects of such infanticide would need to be considered. Such side-effects might include distress for the medical personnel dealing with such cases, societal disapproval directed at (and thus inflicting suffering on) the infants' parents, and the possibility that the generally high respect accorded to life by state and society might be jeopardised – thus leading to widespread suffering, due perhaps to an excessive use of infanticide and euthanasia. Such potential side-effects would need to be considered and, where deemed valid, ought to be factored into utilitarian deliberations on the ethical permissibility/reprehensibly of the proposed infanticide.

Another form of side-effect is the additive effect of individual personal decisions amplified throughout society. For example, future technology may permit the efficient separation of Y-bearing and X-bearing sperm, thus enabling parents to easily choose the sex of their children. Utilitarianism would seem to provide *prima facie* support for the freedom of parents to utilise such technology, since freedom of choice is generally commensurate with happiness. And, if only a small number of parents used sex selection, then the effects on the human population would be negligible. But permitting the wholesale use of this technology (perhaps as a commercial service) might well result in the development of serious imbalances in the sex ratios of human populations. Assuming such imbalances to lead to a reduction in overall happiness, then this side-effect would count against the possibility of making sex selection available for the general population (Dunstan 1988).

A third form of side-effect takes the form of a 'spiral', in which outcomes from a course of action 'spiral' in a self-reinforcing cycle to generate disproportionately strong effects. For example, I have argued elsewhere that several negative outcomes are likely to arise from support (or tolerance) afforded to ineffective, anomalous or implausible 'alternative' medical therapies such as crystal healing, reflexology or homeopathy (Smith 2003a) – see Appendix I. Such outcomes include (a) a diversion of public and private resources towards unconventional therapies at the expense of therapeutic approaches predicated upon logic and evidence, together with (b) a weakening of general commitment towards science-based medicine. These outcomes may interact synergistically and 'spiral' to produce an ethically negative outcome that is significantly worse than the additive effects of each. Thus, considerations of the ethics of permitting individuals their free choice in accessing 'alternative' medical therapies ought to take into account the risk of a 'spiral' side-effect occurring.

Finally, it should be noted that some side-effects may be positive, rather than negative. For example, utilitarians usually support voluntary euthanasia for terminally ill patients (Glover 1977; Singer 1993). The utilitarian's argument, put simply, is that the overall balance of happiness would be increased if voluntary euthanasia were permitted, because patients would have their freedom of choice enhanced and their suffering eliminated. Opponents of voluntary euthanasia may claim that certain side-

effects invalidate this pro-euthanasia position. Such side-effects are similar to those outlined above in the infanticide example, and include distress for the medical personnel, a widespread fear of being pressured into accepting euthanasia, and a reduction in state and societies normally high regard for preserving life, possibly leading to compulsory euthanasia. These side-effects must be assessed and factored into the utilitarian debate, insofar as they are valid. (In my view such claims are scarcely valid and thus fail to torpedo the pro-euthanasia utilitarian argument; however an in-depth discussion and refutation of such claimed side-effects is beyond the scope of this thesis.) But in addition to these negative claimed side-effects, *positive* side-effects from the adoption of voluntary euthanasia are also plausible. Patients might come to fear infirmity and terminal illness less, secure in the knowledge that the medically provided painless death is available as a final release from suffering, if so desired by the patient. Similarly, relatives and friends of terminally ill patients might be greatly relieved by the knowledge that there is a medical way of avoiding end-of-life suffering. And medical staff would no longer have to suffer the stress of effectively prolonging the suffering of terminally ill patients who request voluntary euthanasia.

6.5.2.2 Problems with Utilitarianism

As with all ethical systems, utilitarianism is associated with certain criticisms and limitations:

- Happiness/misery are difficult to quantify
- Utilitarian deliberations can be highly complex
- Drug-induced happiness
- Individual happiness vs. the happiness of many
- Agents vs. actions
- Unlimited moral demands
- Radical, non-intuitive conclusions
- Utilitarianism could be used wrongly

The following sections discuss these problems.

6.5.2.2.1 Happiness/misery are difficult to quantify

It is a fact that happiness and misery are difficult to quantify (Dolan 2001; Easterlin 2003; Haybron 2001; Ng 2000; Van Praag et al. 2003; Zizzo 2002). This is a problem for utilitarianism. Some empirical research suggests that quantification might in future become possible, given major technical improvements in fields such as neuroendocrinology and brain imaging. Meanwhile, it remains the case that degrees of happiness and unhappiness can only be estimated. One way forward may be to employ suitable psychological research methods such as questionnaire approaches. For example, for some genetic disorders, abortion or even infanticide may be considered appropriate by utilitarians in cases where the life of the resulting person was destined to contain more suffering than happiness. In such cases, psychological research into the actual degree of suffering (vs. happiness) experienced by real people with the disorder would be indicated – ideally by soliciting responses from the patients themselves or, where the patients were non-competent, from their parents or carers.

It seems clear that psychological investigation and (more futuristically) neurobiological analysis are tools that should wherever possible be employed in attempts to determine happiness/misery levels. It is also apparent that such approaches are at present insufficiently developed to obtain reliable quantitative data in most ethical dilemmas. However, this is not an insurmountable problem. In many cases, the balance of happiness vs. suffering is quite obvious, without requiring quantification. For example, repeated resuscitation of a terminally ill patient against his instructions, using invasive emergency procedures, would clearly cause serious suffering to the patient, without a counterbalancing generation of happiness. Accordingly, utilitarians would judge it wrong to use resuscitation in such patients, despite the lack of any form of quantitative data on the degree of suffering. Similarly, it is often clear which of two (or more) options will cause the least suffering. To take a very simple example, suppose that a young child will die unless a diseased lung is operated upon, but that the operation is very invasive and will cause the child a great deal of post-surgery

suffering. The choice - to operate or not - should be clear for utilitarians: the operation should take place, because the (unquantifiable) amount of short-term suffering caused by the surgery will be heavily outweighed by the happiness (again unquantifiable) that the child should experience throughout the subsequent years of saved life. Of course, simple examples such as the foregoing give easy answers: in many cases, the balance of happiness and misery is less easy to ascertain, and it may be contentious. However, there is no option in such cases other than to evaluate all relevant evidence and debate the likely effects on happiness/suffering of alternative ways of proceeding. Ethical decisions sometimes need to be reached on the basis of less than perfect information. However, the fact that happiness and misery cannot be accurately quantified does not amount to grounds for a rejection of utilitarianism.

6.5.2.2.2 Utilitarian deliberations can be highly complex

The concern that utilitarian deliberations can be highly complex is directly related to the previous problem. Just as it is true that happiness/misery are difficult to quantify, it is also true that deliberations designed at determining the *likelihood* of increasing happiness (or reducing misery) are frequently fraught with complexity. As discussed in the last section, utilitarian deliberations must consider several factors, including overall sentience, alternative possibilities and side effects. Accordingly, apparently simple cases may upon deeper consideration turn out to be more complicated than was initially apparent. For example, the adoption of an 'opt-out' organ donor register (to replace the 'opt-in' system prevalent in many countries, such as the UK) would seem to be clearly supported by utilitarian considerations: increasing the organ supply would save lives, reduce time spent on dialysis and other 'holding' procedures, and thus increase overall happiness. However, upon reflection there are further questions that need to be considered, including the following: (a) would such a system cause suffering amongst non-consenting relatives? (b) Would the populace be placed in fear of overzealous organ recovery? (c) Would the medical infrastructure be able to utilise the extra organs, or should the money invested in the database be better spent elsewhere (e.g. on research into alternatives to allotransplantation such as xenotransplantation)? Thus, what appeared initially as a clear-cut choice becomes complicated as more possibilities and concerns are raised (of course, such complexity

does not necessarily change the original conclusion: the utilitarian, having weighed up the various new factors, may still be justified in approving an 'opt-out' donor system). As with the difficulty of determining levels of happiness/misery, the problem of complexity does not give cause for abandoning utilitarianism. Rather, it should serve as a warning for utilitarians to approach ethical dilemmas in a maximally reflective and discursive manner, and to obtain as much relevant empirical evidence as possible, in order to decide upon the ethically correct course of action.

6.5.2.2.3 Drug-induced happiness

The problem of drug-induced happiness is often used in attempts to refute utilitarianism (Nozik 1974; Scarre 1996). Opponents of utilitarianism commonly present the following as a knockdown argument:

A. Happiness as the pivotally important feature of utilitarianism.
B. Happiness could be induced simply by adding an appropriate drug to the water supply.
C. Therefore utilitarianism is toppled as a moral system.

But this is an overly simplistic argument. Sentience is an evolved-in features of animals; positive emotional states (happiness, pleasure, ecstasy, etc) serve to signal that all is well, whereas negative emotional states (pain, hunger, terror, etc) serve to spur action (i.e. seeking shelter, locating food, avoiding danger, etc). In the absence of this correlation between real-world situations and emotional states, sentient beings would be unable to survive. If a truly effective happiness-inducing drug were added to the public water supply, the populace would presumably remain happy while food ran out, while disease spread and while children were neglected; in the presence of such drug-induced happiness there would be no impetus to pursue misery-reducing measures. Thus, the drug-induced happiness would be very short lived, as the population dwindled. Accordingly, utilitarianism implicitly rejects as unethical such drug-induced happiness, on account of the long-term reduction in happiness entailed by the use of a happiness drug.

Of course, it is possible to envisage a futuristic situation in which some form of effective machine (of global magnitude) supplies all the material needs of humanity, while humans exist in a state of drug-induced happiness. Aside from issues of implausibility, the major problem for this scenario is that it converts people from individuals possessing the potential to act as moral agents into passive beings unable to contribute to ethical decisions. Thus, the adoption of such a 'utility machine' would imply the end of any possibility of ethical progress. Accordingly, it appears that, instead of undermining utilitarianism, happiness drugs and 'utility machines' are themselves invalidated on the utilitarian perspective.

Although it can be effectively argued that the notion of drug-induced happiness fails to topple utilitarianism, it need not be concluded that drug-induced happiness *per se* is of no utility. Consider somebody in the final stages of terminal illness, or a maimed battlefield victim, or a post-operative patient: such persons may well benefit from – and strongly desire - a 'happiness drug', if only to relieve their pain or misery. And indeed such medication is commonly offered to such persons, in the form of opiates and anxiolytics. The difference between such drug-induced happiness and the 'universal' form rejected above lies in the limited, temporary and voluntary nature of the former compared with the global, permanent and compulsory nature of the latter.

6.5.2.2.4 Individual happiness vs. the happiness of many

The potential conflict between the happiness of particular individuals and the general happiness presents a difficulty for utilitarianism (Glover 1977; Mill 1871; Parfitt 1984; Scarre 1996). If the general happiness is what counts, then it seems to follow that the happiness of individuals may be sacrificed for the happiness of a greater number. Utilitarianism might seem to favour a number of repugnant actions or situations such as compulsory sterilisation of individuals at risk of transmitting a serious genetic disorder, and permanent quarantine or even death for those infected with HIV. In such cases, the benefits to the majority - prevention of disease and avoidance of attendant suffering – would appear to outweigh the costs borne by the minority. Thus, the charge is that utilitarianism is inherently unfair, unjust and lacking in respect of individual persons.

However, the question needs to be asked: Does utilitarianism actually imply such reprehensible courses of action? I suggest the answer is emphatically no. In practice, running roughshod over individual life and liberty is likely to result in a state of fear and discontent, and hence destroy normal society. Since such a disastrous outcome is clearly one of great disutility, utilitarianism could not support repugnant actions against minorities or individuals, even where a *prima facie* case may be made in favour of such actions on the grounds of the general good. Thus, it would be highly simplistic, or a distortion, to suppose that utilitarianism runs contrary to common notions of fairness, justice and respect.

Although utilitarianism does not imply the blatant sacrifice of the few for the good of the many, some utilitarian ethicists have suggested that the utilitarian approach ought to be tempered by an additional principle beyond the happiness/misery calculus, namely *respect for autonomy* (Glover 1977). This principle is particularly salient in the context of decisions concerning individual people. The principle of respect for autonomy holds that a competent, informed person (such as an adult patient) ought to have the freedom to choose whether or not to consent to a proposed course of action (such as a medical procedure), regardless of the associated objective benefits (to the greater happiness *or* to the individual concerned). However, it is worth noting that the principle of autonomy is itself derivable from utilitarian considerations because, once again, in the long run it would be detrimental to the general wellbeing if the autonomy of individuals were to be routinely disregarded.

Although I have concluded that utilitarianism is not in fact the unfair and unjust system claimed by its opponents, nevertheless it should be noted that there may be rare occasions when it would indeed be better for the few to suffer in order to benefit the vast majority. To take a hypothetical situation: suppose (a) that a highly infectious new disease is poised to cause widespread human death and morbidity, (b) that scientists have produced a vaccine that is able to prevent the disease and (c) that the effectiveness of the vaccine depends upon 95% of the population being vaccinated. In this case, a strong utilitarian argument could be made in favour of compulsory vaccination, despite the costs to the minority who refuse to be vaccinated (i.e. loss of autonomy and occasional adverse reactions to the vaccine). In extraordinary cases

such as this, the arguments in favour of acting to secure the general good would appear to be difficult to refute. Certainly, by permitting some relaxation of normal mores of behaviour *in extremis,* utilitarianism stays in close approximation to common sense morality.

A final consideration in the context of the happiness of the few vs. the happiness of many concerns the following scenario: For any large population of people, all with happy lives, there could in theory be some much larger population that would contain a greater quantity of happiness, even though its members all had lives that were *only just* happy. Given the relative amounts of happiness involved, utilitarians would seem obliged to judge the latter population as preferable to the former – but this judgement clashes sharply with our intuitive sensibilities (Parfitt 1984). This problem turns out to be rather intractable, and no solution appears to have been published to date. Fortunately however, the importance of this problem is primarily theoretical rather than practical, and it is more relevance for population ethics than it does for bioethics. Thus, a detailed consideration of this problem lies outside the main focus of the present discussion. Nevertheless, I have constructed a provisional response to this problem – see Appendix II.

6.5.2.2.5 Agents vs. actions

A separation between agents and actions is often implied by utilitarianism. Such a separation has drawn strong criticism from disparate non-utilitarian ethical creeds (Comte-Sponville 2003; Scarre 1996; Williams 1993). In common morality, and in most non-consequentialist ethical systems, the *character* of the person facing an ethical decision (the 'agent') is deemed important. Indeed, some ethicists view the *improvement* of the agent's character as being the prime objective of morality. Related to this idea is the notion that the *motivation* for performing an ethical act is highly salient. However, utilitarianism generates scenarios in which good consequences can (a) flow from the actions of agents of poor character, (b) result in no improvement in the agent's character and (c) be borne by motivations that are clearly negative. For example, suppose that a very rich - but also very greedy and selfish - businessman decides that he needs to gain local approval, in order to increase

his chances of winning a lucrative property development contract. Accordingly, and in the absence of any genuine concern for the plight of impoverished people, he cynically makes a highly public donation of a large sum of money to a Third World charity. The direct (ethically relevant) outcome from this action is highly positive, in that the lives of several impoverished children in the Third World are saved through a vaccination program paid for by the donated money. Thus, the selfish businessman's action of donating the money ought to be condoned by utilitarians, given the positive consequences. However, the businessman's character was not praiseworthy before he made the donation (greediness and selfishness are hardly commendable attributes), nor has his character improved as a result of making the donation (he has simply become a little more expert at satisfying his greedy ends), and his cynical motivation can hardly be admired. Accordingly, there appears to be a problem for utilitarianism. The problem is one of inconsistency between (a) the ethical status of an agent, and (b) the ethically relevant consequences of an agent's action.

This difficulty for utilitarianism may be countered in a number of ways, with arguable degrees of success. Firstly, it can be argued that *all* charitable giving is inherently selfish, because the motivation of the giver is ultimately one of self-interest; on this analysis, even the most apparently altruistic acts of charity are selfish because such acts will be motivated (at least) by the desire to become a better person. If this analysis is correct, then utilitarianism can hardly be singled amongst moral systems for failing to consider the character and motivation of the agent. However, I am not persuaded by this argument. Assuming (as all moral systems do) that it is actually possible to be a morally praiseworthy agent, then the desire to be a moral agent would appear to qualify as an ethically admirable aspiration.

A second possible solution to the agents vs. actions problem may be obtained from the observation that utilitarian acts are not *necessarily* incompatible with common conceptions of ethical praiseworthiness in an agent. Very often, actions that have good consequences are associated with agents of good character, and with the improvement of character. Indeed, persons of good character may well deliberately choose actions based on clearly utilitarian grounds (for example, the donation of money to good causes *motivated by* the desire to save lives and alleviate suffering).

Another possible resolution to the issue of agents vs. actions is simply to attempt a *reclassification.* Instead of categorising the inconsistency as a *problem,* perhaps it should instead be considered simply as a *natural feature* of utilitarianism. This might be formalised thus: "utilitarianism implies an unavoidable division between agents and actions". Utilitarianism would then be left to stand or fall on its other merits and demerits. Of course, in the case of moral systems in which character and motivation are intrinsically *inseparable* from consequences, or in which character and motivation are the sole factors deemed ethically salient, the 'natural feature' in question dictates an automatic rejection of utilitarianism.

The foregoing attempted solutions notwithstanding, the question may be asked: In the context of medical ethics, is the issue of agents vs. actions a major consideration? In medical ethics, the agents must (or should) always remain at some distance from the actions involved. This contrasts with *personal* ethics, in which matters of character and motivation arguably form a central part. In medical ethics, the agents are typically members of an ethics committee (within a hospital, university, or government, etc). The duty and raison d'être of the ethics committee is to make the best possible ethical decisions – for example, to decide whether a proposed medical experiment should be permitted, to decide whether a new medical procedure is safe enough to be offered to children, and so on (Glasa et al. 1996; Rosner 1985). But, unless a conflict of interest is involved, the personal character of each committee member is of no relevance: committee members may be philanderers, be ruthless in business transactions, be spiteful, or never donate to charity – such attributes do not matter as long as the committee reaches appropriate ethical decisions. Similarly, the function of the ethics committee obviously does not include any requirement to improve the character of the committee members. And, although it may be the case that many ethics committee members are motivated to participate through a desire to ensure that medical decisions are ethically acceptable, such motivation is not a prerequisite for membership. Some ethics committee members may be serving different, perhaps less admirable motivations: for example, some members may be seeking to improve their professional standing or chances of gaining promotion, and others may be present simply out of professional duty. However, assuming (a) that the committee members are competent and (b) that they participate effectively, then their motivation for being ethics committee members is immaterial. In conclusion, the agents vs. actions

criticism of utilitarianism *per se* does not cause a problem in the context of medical ethics, and nor therefore does the criticism represent a significant problem in the case of the present thesis.

6.5.2.2.6 Unlimited moral demands

Utilitarianism has been strongly criticised on the grounds that it makes unlimited moral demands on the individual (Brock 1982; Scheffler 1982; Wolf 1982). Because utilitarianism appears committed to maximising human wellbeing regardless of the identities of individuals, the moral agent ought to place the general wellbeing above his own personal concerns. From this, it seems to follow that a moral agent ought to donate all his money and time to good causes (save a bare minimum for basic survival needs). Indeed, an agent would appear to be obliged to sacrifice his life or endure extreme torture in cases where so doing would improve the general happiness. Thus, it has been argued that utilitarianism makes such heavy demands on individuals that it implies a *reductio ad absurdum* of the theory. The criticism, put simply, is that moral sainthood is incompatible with normal human behaviour. To the extent that this criticism may be valid *per se,* it does little to undermine utilitarianism in the context of medical ethics. As with the previously considered question of 'agents vs. actions', the personal virtues and moral attributes of the decision-makers in medical ethics are normally of no consequence: the only important question is whether the ethics committee is reaching appropriate ethical decisions. Accordingly, any in-depth exploration of the problem of unlimited moral demands lies outside the main focus of this thesis. This problem is nevertheless an important one for utilitarianism *per se.* The utilitarian agent involved in medical ethics may wish to avoid a situation of 'bad faith' in terms of a conflict between professional ethics and personal ethics. I have previously published a response to the problem of unlimited moral demands (Smith 2002a) – see Appendix III.

6.5.2.2.7 Radical, non-intuitive conclusions

This problem concerns the fact that radical, non-intuitive conclusions are sometimes implied by utilitarianism. In contrast to some ethical systems, utilitarian deliberations often dictate courses of action that conflict with 'commonsense' morality (Kuhse and Singer 1999; Singer 1993). For example, a utilitarian argument can be made in favour of using pigs for xenotransplantation, on account of the likelihood that the happiness resulting from more transplant patients being treated will outweigh the suffering experienced by the pigs. However, similar lines of utilitarian reasoning suggest the conclusion that the intensive farming of pigs for meat should be *condemned*, on account of the negative overall happiness involved (can happiness from eating bacon really outweigh the animal suffering inherent in intensive pig production?). However, the fact that radical conclusions are sometimes generated cannot be a good reason for rejecting an ethical theory. We ought not dismiss utilitarian conclusions simply because they happen to be radical or non-intuitive (unless the conclusions generated were actually *repugnant* or genuinely *ridiculous*). Indeed, if it is accepted that moral systems ought to be rejected unless their conclusions match those of common-sense morality, then ethical systems in general (indeed all ethical discourse) would have no function. Yet the very thing that is clearly required is some form of ethical system (and associated ethical discourse) that can help us to deal with the sorts of difficult, complex and novel problems that are raised by cutting-edge biomedical technologies. I take it as axiomatic that common-sense morality is inadequate to serve such a function.

6.5.2.2.8 Utilitarianism could be used wrongly

A final problem is the possibility of misuse (Glover 1977; Scarre 1996; Williams 1993). Utilitarianism may indeed be used wrongly, either through error or through deliberate misuse. For example, a simple failure to weigh-up all possible side-effects may lead us to condone an apparently beneficial fish transgenesis experiment, where a more thorough analysis would have pinpointed a high risk of serious suffering of African coastal peoples through damage to their fish stocks should the transgenic fish

escape into the wild. More deliberate misuse of utilitarianism would include (for example) the acceptance of major animal suffering in order to produce yet another mild headache cure (some instances of misuse may be disguised by the slogan "the end justifies the means" – but this slogan is a simply an inaccurate corruption of utilitarianism, employed as a rhetorical device). However, the risk of misuse fails to count against utilitarianism: almost any idea - regardless of its nature (scientific, religious, metaphysical, etc) - may be misinterpreted by the ill-informed, or dishonestly distorted by those with their own agenda. All that can be done is to guard against such mistakes and misuses.

6.6 Conclusions

The author of this thesis sees utilitarianism as a developing branch of ethics that holds great promise for issues raised in medical ethics in general, and in cutting-edge biomedicine in particular. Utilitarianism is certainly not free from problems. However, I suggest that these problems can be answered by, or accommodated within, utilitarianism. It seems reasonable to claim that utilitarianism, if used carefully and honestly, offers an effective (although imperfect) guide to ethical decision-making.

At the very least, utilitarianism represents one valid contender amongst several ethical approaches that may be used in the context of medical ethics. Accordingly, attempts to apply core principles of utilitarian reasoning to particular cases should be seen as intrinsically valid and potentially useful. This is the approach taken in respect of the bioethical aspects of this thesis.

Chapter 7: Human Germline GM – Bioethical Considerations

7.1 Preface

This chapter presents an original application of core utilitarian principles to the ethics of human germline gene therapy. The nature of this chapter differs from that of the science-based chapters (Chapters 2-5) in the same ways as does the previous chapter (Chapter 6). Thus, references are minimal, and first person language is present. The reasons for this apparent (but necessary) mismatch between the scientific and the bioethical sections are the same as those applying to the previous chapter (see 6.1).

7.2 Safety, Effectiveness and Consent

Safety is a key concern for all therapeutic modalities, conventional or genetic (Beauchamp and Childress 2001; Livadas 2002; Subramanian and McCullough 1987; Veatch 1984). For the utilitarian, questions of safety involve balancing risks against benefits. Given the inevitable fact that no medication or medical procedure can be absolutely safe, it is difficult to see any plausible alternative to the risk-benefit assessment approach to safety issues inherent in utilitarianism. This section considers the related issues of safety, effectiveness and consent from an ethical standpoint, with issues arising from germline gene therapy being contrasted with those arising from somatic gene therapy (Section 5.2).

While safety issues apply to both somatic gene therapy and germline gene therapy, the matter is simpler in the case of the former. From the utilitarian perspective, potential health risks give rise to the main ethical issues associated with somatic gene therapy. In the case of possible harm to adult patients, the matter is relatively straightforward: consent must be sought, with the patient's decision facilitated by an explanation of the risks involved. Thus, the decisions made by individual patients to accept or decline somatic gene therapy are essentially the same as those decisions made in the context of conventional medicine. By contrast, germline gene therapy has safety implications that apply to future people, as opposed to existing patients.

A related distinction also exists between somatic and germline therapies in regard to the issue of consent. Whereas in the former case the patient is competent to decide whether to give or withhold consent, in the latter case the 'patient' can play no part in any such decision. This distinction may at first sight appear to be unique to human germline gene therapy. However, this is not entirely so.

In many genetic and other serious conditions, the patient is often a child. In such cases where somatic gene therapy is under consideration, ordinary medical ethics provides a guide to action in such cases: the ability of the child to balance the benefits and risks from the proposed therapy must be determined. In keeping with generally established medical practice, the assessment of the child's ability to give/withhold consent should be determined on a case-by-case basis (rather than simply by chronological age), and the previously mentioned *principle of autonomy* should be applied wherever it seems likely that the child is able to make a rational decision. However, it should be noted that many of the serious genetic conditions potentially amenable to somatic gene therapy often kill or seriously damage patients in their first months or years of life. Thus, consent difficulties are expected to occur more frequently with somatic gene therapy than with ordinary medical approaches. Consequently, this fact brings somatic gene therapy closer to germline gene therapy in terms of consent issues.

Indeed, as a generalisation, conceptually there exists an inverse correlation between the age of the patient and the probable effectiveness of somatic gene therapy. Accordingly, the foetus is often viewed as an ideal developmental stage for somatic gene therapy. In addition to permitting treatment prior to irreversible disorder-induced damage, foetal somatic gene therapy has a number of potential advantages compared with later-stage approaches (Coutelle and Rodeck 2002; Kaplitt et al. 1998). These advantages include: (a) better transgene uptake efficiencies due to the high percentage of rapidly dividing cells populating the foetus (as discussed previously, some gene transfer approaches do not function well with non-dividing cells); (b) the possibility of generating whole-tissue/organ alterations by genetically altering a small number of appropriate clonogenic cells (such cell-types are a feature of early foetal development); and (c) the avoidance of an immune response against transgene vectors due to the preimmune status of the early foetus. Thus, given the scientific advantages associated with the foetus, somatic gene therapy may well come to be frequently

targeted at the unborn child. In foetal somatic gene therapy there can obviously be no consent from the 'patient' affected (beneficially or detrimentally) from the treatment. Again, this fact brings somatic gene therapy closer to germline gene therapy in terms of consent issues.

From the consequentialist standpoint advocated in this thesis, foetal gene therapy should in principle be permitted against serious, otherwise untreatable disorders in cases where the following criteria can be met: (a) where there is a demonstrably high probability of a successful clinical outcome, and (b) where the risk of serious iatrogenic damage is very low. If these criteria cannot be met, the choice then becomes one of allowing a child to be born with a serious disorder, or terminating the pregnancy (this choice is essentially parental, and the associated ethical considerations are outside the scope of the present discussion). However, if human germline gene therapy was available, then this would represent an additional option for the parents: instead of conceiving a foetus that would be treated with somatic gene therapy (or aborted), germline gene therapy would avoid the medical problem occurring in the first place. Thus, from a bioethical perspective, human germline gene therapy is very similar to foetal gene therapy to the extent that the 'patient' is unavailable to give or withhold consent.

Because *future* people are the subjects of foetal gene therapies and germline genetic therapies, the associated ethical considerations go beyond issues of patient consent. Much moral discourse exists on the metaphysical questions arising from the possibility of foetal gene therapy and germline gene therapy. For example, does a future person have moral *rights?* Is an unborn child entitled to an 'open future' rather than one 'designed' by others? Would the genetic alteration of a future person constitute an assault on the 'humanity' of that individual? Such questions represent valid areas for professional moral philosophers and theologians to pursue. However, from a utilitarian perspective, I suggest that – if progress is to be made - the ethical focus should be upon the matter of *interests.* Thus, it may be argued that a proposed foetal or germline gene therapy would be (or would not be) in the *best interests* of a future person.

It would be a mistake to assume that an interests-based approach is unique to gene therapy. In the case of prenatal diagnosis, it is necessary to decide whether a foetus harbouring a medical disorder should be aborted or allowed to live. Similar considerations apply to questions of medical intervention in the case of patients in persistent vegetative comas. And decisions have to be made on behalf of young children who are insufficiently mature to give or withhold consent to medical procedures. In each of these cases, a judgement must be made as to what is likely to be in the best interests of another person. Such judgements are often very difficult to make, and require a good deal of medico-scientific understanding coupled with careful reflection. Nevertheless, there is no obvious alternative way to reach decisions concerning medical interventions that will affect people who cannot give or withhold consent. Accordingly, an interests-based approach for decision-making in the context of germline gene therapy would not differ fundamentally from the approach that is already well-established in conventional medical ethics.

There is however a special feature of germline gene therapy that complicates ethical decision making. It is the issue of *future generations* of people. In medical decisions concerning foetuses, children or comatose patients, the effects of medical intervention will be limited to the individuals concerned. By contrast, germline gene therapy implies genetic changes that would be transmitted to all descendant generations arising from the 'treated' individual. Furthermore, once incorporated into the germline of one individual, a transgene sequence could have the potential to 'spread' (i.e. increase in frequency) throughout the entire population.

It is this issue of having permanent (and potentially damaging) effects on future generations that makes germline gene therapy especially contentious from an ethical perspective. Given its potential magnitude of effect, one may be tempted to rule germline gene therapy *ultra vires,* for fear of causing unalterable and undesirable changes to humanity *per se.* However, there are good reasons to believe that such an absolutist response is one of excessive caution.

Firstly, germline gene therapy should not be singled-out as the only factor able to alter the genetic constitution of future generations. As mentioned previously, medical X-rays on occasion undoubtedly induce mutagenic changes in patients' germline DNA.

Environmental factors, ancient and contemporary, damage germline DNA. Moreover, germline mutations are a frequent and inevitable occurrence *in vivo*, regardless of environmental factors. Thus, altering the human germline would hardly represent a new, unparalleled occurrence.

Secondly, the fear of inducing unalterable changes is valid insofar as deleterious (accidental) changes are concerned. However, medically sought germline alterations would be aimed towards beneficial effects – for example, the repair of an oncogene, or the knockout of a dominant disease gene, or the addition of a missing gene function. From an ethical perspective, such benefits ought to be given weight. Thus, for any proposed germline gene therapy, if the benefit (e.g. a large decrease in cancer risk through the repair of an oncogene) were to outweigh the risks (e.g. insertional mutagenesis), then it would be difficult to ethically defend proscription of the therapy concerned. Moreover, the issue of affecting future generations would in such cases weigh *in favour* of germline gene therapy, since many people (i.e. the descendants of the 'treated' individual) would stand to benefit from the therapy.

Thus, on the utilitarian view, the ethical issue becomes a scientific one: namely, the scientific assessment of (a) efficacy and (b) risk of accidents. Clearly, proposed germline therapeutic approaches would need to be individually (and very rigorously) assessed in terms of (a & b), on a case-by-case basis.

The main potential health risks associated with germline gene therapy are: (a) mutagenic (including potentially carcinogenic) effects arising from transgene integration; and (b) developmental abnormalities arising from the use of specialised cells such as ESCs and reconstructed embryos (in NT approaches). As discussed previously, all the presently available random integration forms of gene transfer (pronuclear microinjection, RVVs, SMGT) are associated with significant risks of genetic damage. The question is, do such risks outweigh the benefits? In pronuclear microinjection and SMGT, genetic damage is expected at a frequency of less than 2%, with most occurrences of such damage expected to be of minor consequence to health. This level of risk may appear tolerable, especially if viewed as the cost of avoiding a devastating monogenic disease such as CF or haemophilia. However, as previously discussed, embryo pre-screening can be used to avoid monogenic conditions

potentially amenable to human germline gene therapy. Given that pre-screening is not associated with genetic damage, it appears inescapable that the risk of genetic damage from pronuclear microinjection or SMGT effectively precludes the use of these techniques (at least in their present form) in the context of human germline gene therapy. It would be unethical to risk genetic damage by using such forms of human germline gene therapy instead of pre-screening. The case against RVVs would be even stronger, considering the additional risks associated with of such agents - integration into oncogenes and reactivation within the host genome.

Gene targeting would avoid the risks of insertional damage. However, NT transgenesis is the only gene targeting approach presently applicable (in principle) for humans. However, because NT is associated with a very high level of disease and debility in the first generation, this approach is ruled out on safety grounds. Again, it would be unethical to risk developmental (epigenetic) damage by using NT-based human germline gene therapy when prescreening could deliver the same results with no such risks. Thus, it would be unethical to carry out germline gene therapy where prescreening is a safer alternative. Given the current status of gene transfer technology, the conclusion must be that human germline gene therapy ought not be carried out at present.

However, this negative conclusion is not inviolable. As the underlying progresses, gene transfer techniques will undoubtedly become more effective. It is impossible to envisage the ultimate degree to which GM methodology will progress, much less to set a timescale for progress. Nevertheless, there are no fundamental reasons to doubt that significant technological improvements will be made. It is salutary to reflect on the relative infancy of genetic modification science: the first use of GM (in prokaryotes) occurred in the early 1970s; mammalian transgenesis was first accomplished in the early 1980s; and the first (somatic) human gene therapy trials began in the early 1990s. Thus, in little over 30 years there has been a revolution in GM science and technology. The following 30 years will undoubtedly see further advances. If GM technology develops to the point at which human germline gene therapy would be safer and more efficient than alternatives such as prescreening, then utilitarians would have no safety-based grounds for objecting to human germline gene therapy.

7.3 Public Acceptability

Human germline gene therapy may be objected to on the grounds that it appears to be unacceptable to (at least some sectors of) the public. In this regard, a distinction needs to be made straight away between human germline gene therapy using present gene transfer technology, and human germline gene therapy of the future. The public would be right to object to any proposal to perform human germline gene therapy using present technology: as discussed above, current gene transfer methods are simply too risky (in comparison with pre-screening) to be used as the basis for attempted germline gene therapy on humans. By contrast, safety concerns would not be valid grounds for objection in the futuristic (but plausible) scenario where future gene transfer techniques had advanced to the point at which the benefits outweighed the risks. In this future context, if public disapproval of human germline gene therapy existed, such disapproval would have to be based upon considerations that go beyond the question of safety vs. effectiveness. Some contenders as valid arguments against (safe and effective) human germline gene therapy are discussed in the remaining sections of this thesis. However, there is a notion that 'public acceptability' is an ethical entity in its own right. In this respect, should 'the public' (or a majority thereof) not wish to have human germline gene therapy permitted, then this would be seen (by some) as having ethical force against human germline gene therapy.

There have been many instances of scientific innovations against which 'the public' – at least as represented by the popular media – have taken a negative stance. Recent examples at the time of writing include the MMR vaccine controversy and the issue of GM crops. I suggest that such issues ought to be decided by debate based on scientific knowledge and rational argument: if there are scientific and/or ethical objections to a particular medical procedure or technological innovation, these should be brought forward for open discussion by all interested parties. I suggest that it is erroneous to view 'public acceptability' as a form of ethical black box, with its own validity that transcends the many arguments for and against whichever issue is at stake.

Thus, it should be open for any member of the public to raise objections to human germline gene therapy. However, should there be widespread public apprehension of

human germline gene therapy (as has occurred in the UK with GM crops), this fact of public opinion should not of itself count as a strong ethical argument against human germline gene therapy. Of course, utilitarianism is generally supportive of popular democracy and the underlying autonomy that democracy implies for each member of the public, and in this respect utilitarianism might permit public opinion to prevail, even if the basis for such opinion was irrational. This would be justified on utilitarian grounds in cases where serious civil strife, societal breakdown or terror amongst the populace would be a likely outcome of refusing to allow the popular will to prevail – assuming that the potential benefits (for example improved resistance to particular diseases amongst those treated by human germline gene therapy) were smaller than the disutilities likely to arise from ignoring the public will.

In other words, the possible side-effects of human germline gene therapy would need to be taken into account, and such side effects might include public upset. One form of side-effect that is of particular importance to utilitarians is the intuitive response. I have previously argued that intuitive responses are poor guides to ethical decision making, and are incompatible with utilitarianism (Section 6.4.1). Nevertheless, intuitive responses exist as real features of human psychology and hence of the public at large. If a large number of people found human germline gene therapy difficult to palate, despite valid arguments in its favour, this would count against permitting human germline gene therapy. This is so because ignoring a deep-seated intuitive response would cause psychological upset and thus be a disutility. A contemporary example illustrates this: late abortion or even infanticide may be justified on utilitarian grounds, in cases involving untreatable disorders that would seriously reduce the quality of life (Glover 1977; Singer 1993). To a utilitarian, such termination of life would be supported by several considerations including: (a) the reduction in potential suffering for the afflicted individual and the parents; (b) the lack of 'personhood' of foetus or infant; and (c) the possibility of 'replacing' the terminated life with another. Notwithstanding such strong utilitarian arguments, it remains the case that many people find late abortion and – in particular – infanticide intuitively repugnant. It may be asking too much of the public to accept infanticide, despite the arguably good reasons in its favour, in the face of strong intuitive reaction against it. Thus, the best consequences would come from a proscriptive position, if it were likely that the

merits of allowing infanticide (a reduction in disease-related suffering) would be outweighed by the demerits (serious psychological upset and possible societal strife).

Would there be a widespread, deep-seated intuitive reaction against human germline gene therapy, of similar magnitude to the probable response against infanticide? It is difficult to be provide a precise answer, because public opinion is subject to cultural influences: for example, vociferous protest groups may be able to hijack the debate and sway the public against accepting human germline gene therapy. A pertinent example here would be GM crops, which have been largely accepted in the USA but rejected by a large proportion of the UK populace. However, whereas humans are probably innately repulsed by the idea of killing infants (although even here culture plays a part: in some communities, infanticide of disabled infants has been an enduring cultural norm), it is less clear that we should be similarly 'programmed' against human germline gene therapy. One reason for employing non-intuitive deliberations to ethical problems is to circumvent the fact that our intuitive response 'system' has not evolved to cope with novel issues such as human germline gene therapy: thus, there is no reason to assume a strong intuitive response against human germline gene therapy. Moreover, human germline gene therapy has no obvious parallel in ordinary/historic human experience. This is in contrast to some other modern issues: for example, while surgical late abortion is relatively new to human experience, it involves fundamental elements (blood, infants, death, etc) that evoke deep-seated intuitive reactions in humans. Similar fundamental elements are absent from any plausible account of human germline gene therapy. Furthermore, it should be clear even to non-reflective persons that human germline gene therapy is a technology intended to have positive outcomes – i.e. its raison d'être is to prevent disease. Thus, because no counterintuitive logic is required, it should be relatively easy for human germline gene therapy to gain acceptance even amongst unsophisticated persons. This contrasts with the example of infanticide, which involves the counterintuitive notion that killing infants may be ethically desirable.

A small body of research has been published that investigates the views of the public towards gene therapy (Hedgecoe 2004; Macer 1992; Macer 1994; Macer et al. 1995; Scully et al. 2004). In general, people perceive both benefits and risks from genetic manipulation. Further research would be very welcome, especially in regards to

germline (cf. somatic) gene therapy. But it would be surprising if a serious deep-seated intuitive response against human germline gene therapy were to be discovered, considering the foregoing discussion.

7.4 Cost and Access

Current germline GM technology is expensive. For example, the cost of producing one (founder) transgenic sheep by pronuclear microinjection is around £30,000 (Wall 1996; Wall et al. 1992). If the present technology is improved incrementally to the point of being sufficiently safe and effective to permit human germline gene therapy, its high cost would restrict access. In non-state funded healthcare systems (such as in the USA), this would probably mean that only financially better off individuals would be able to afford human germline gene therapy. In public healthcare systems (such as in the UK), some form of rationing would probably be required. And human germline gene therapy would probably be totally unavailable for the citizens of poor countries.

Of course, such a situation is invidious. Utilitarianism (in common with many ethical systems) is supportive of the widest possible access to medical goods: restrictive access would (a) limit the decrease in suffering available from disease-preventing measures, and (b) increase discontent and unhappiness amongst those unable to access medical goods. However, it would be erroneous to isolate human germline gene therapy as a special case insofar as cost is concerned. Many medical procedures (such as essential transplant surgery) are extremely expensive, as are many life-saving drugs (such as anti-HIV medication). It would be difficult to construct a utilitarian argument in favour of prohibiting the development or use of an expensive medical approach solely on grounds that the high cost would limit access. The reasons for this are twofold. Firstly, the development of initially expensive medical technology may pave the way for the evolution of cheaper forms of the technology. Secondly, even if only a small number of people can be treated by an expensive medical technology, these persons would experience an increase in happiness; thus a positive contribution would be made to the overall amount of happiness in the world.

Of course, there is a strong utilitarian argument in favour of spending a limited budget on simple, cost-effective measures that benefit many people, rather than spending it on expensive, high-tech measures that benefit a smaller number of people. For example, suppose a poor country has £1,000,000 to spend on healthcare: this money would probably be better spent on a child immunisation program than on a cardiac surgery unit. The same argument would apply to decisions to spend limited funds on human germline gene therapy versus (say) a clean needle for hospitals program. However, it is important to note that this type of argument says nothing whatsoever about the intrinsic ethical desirability/reprehensibility of human germline gene therapy.

A major problem would arise if human germline gene therapy came to be used not simply to avoid particular genetic diseases, but to endow genetic advantages. Such advantages might include cancer resistance, increased life-span, physical prowess, enhanced IQ, etc. It is debatable whether such 'enhancements' to the human genome are desirable *per se;* but it seems clear that should access to such enhancements come to be restricted to rich individuals (and hence their children), then societal harmony would be severely jeopardised. Accordingly, the use of human germline gene therapy for genetic enhancement of a subgroup of humans would not be supported by utilitarian morality. The issue of genetic enhancement – and its possible corollary, eugenics – is discussed in more detail later in this chapter.

The above discussions are based on the possibility of human germline gene therapy becoming technologically feasible by incremental improvement in current technology. An alternative scenario, and one that would be equally consistent with the general history of scientific progress, is that technological improvements *revolutionise* gene transfer such that human germline gene therapy becomes not only safe and effective, but also inexpensive. It is impossible to envisage exactly what such human germline gene therapy would look like, but a few points are worth making. Firstly, there would have to be no need to remove/return eggs or embryos from/to the body, since the necessary steps are intrinsically expensive in terms of trained staff and facilities. Secondly, there would have to be no need to perform transgene addition to individual cells, since the required procedures and equipment are again inherently costly. These features of future low-cost human germline gene therapy dictate that some form of

SMGT would have to be involved, with the use of a high-efficiency vector to deliver a targeting transgene to sperm via either *in vivo* injection prior to intercourse or *in vitro* exposure prior to AI. If such improvements are ever realised, then ethical concerns over the costs of human germline gene therapy and access to it would largely disappear.

7.5 Human Embryos

The use of human embryos in biomedicine generates controversy (Chu 2003; FitzPatrick 2003; George 2004; Karpowicz et al. 2004; Williams et al. 2003). This is unfortunate for human germline gene therapy, since research with human embryos would be an unavoidable precondition to the establishment of effective human germline GM methods. Additionally, most presently available gene transfer methods entail the loss of embryos during the GM process. A detailed discussion of the ethics of human embryo research is outside the scope of the present thesis. Suffice it to say that utilitarianism does not place special ethical importance on the human embryo. To put the utilitarian case simply: only beings that have interests can be harmed, and having interests presupposes sentience; embryos (in contrast to late-stage foetuses, children and adults) clearly are non-sentient, and therefore no interests are defeated if embryos are experimented upon. Destroying a human embryo does not kill a person, rather it prevents a person from coming into existence. The killing of a human embryo harms no person.

I suggest that the onus should be on those who oppose human embryo research to make convincing arguments in support of their case. Much is at stake; not only human germline gene therapy, but biomedical research in general will be undoubtedly be seriously retarded if research on human embryos is – as it is in the USA – prohibited. Of course, many of those who are implacably opposed to human embryo research are motivated by religious beliefs, and regrettably there may be little hope of taking the argument forward with such persons.

Pre-screening as an alternative to human germline gene therapy has already been discussed, with the conclusion being drawn that pre-screening is preferable to human germline gene therapy given the safety problems and effectiveness limitations of presently available GM technology. However, suppose human germline gene therapy became equivalent to pre-screening in terms of safety and effectiveness: which approach would be preferable? It has been suggested that human germline gene therapy may be preferable to selective abortion or discard *"shows greater respect for children and adults who are afflicted with disease or disability"* (Walters and Palmer 1997). Utilitarianism can have no special objection to selective abortion or discard; however, the issue of side-effects would tend to support the viewpoint expressed in the above quote. Thus, should human germline gene therapy develop to the point at which it is on a par with pre-screening in all ethically relevant respects (including safety, effectiveness, cost), then it would probably be best to favour human germline gene therapy.

7.6 Sequence Alteration

Genetic modification entails the deliberate alteration of genetic sequences within the genome. In nature, the same fundamental process of sequence alteration occurs as a result of natural selection and associated evolutionary mechanisms. Similarly, the process of sequence alteration occurs in the selective breeding of domesticated animals and plants. In terms of sequence alteration, the only significant difference between genetic modification and genetic selection is that the former process is very much faster that the latter. Thus, ethical objections against gene therapy based on a notion of the intrinsic wrongness of sequence manipulation would be sustainable only as a subset of a much broader objection to all forms of *deliberate sequence alteration* (DSA) (Smith 2002b). A coherent anti-DSA argument would entail the approval or acceptance of sequence alterations occurring naturally (from evolution) and the rejection of deliberate forms of alteration (breeding and genetic modification). Thus, to assume an ethical stance against DSA would be to subscribe to an unsubtle 'naturalistic fallacy': in other words, DSA would be deemed morally unacceptable on the grounds that it is 'unnatural'. One need only consider (a) undesirable 'natural'

events (e.g. infection by HIV) and (b) beneficial 'unnatural' events (e.g. the use of anaesthetics) to see the fallacious nature of 'naturalistic' arguments.

Additionally, it is difficult to see how any form of rational ethical argument could be made against DSA in respect of its actual historical consequences, considering the vast expansion of thriving humanity that would not have been possible without centuries of selective breeding of domesticated animals and plants. Of course, negative applications of DSA are perfectly possible: for example, a terrorist regime employing genetic modification to worsen the effects of a biological weapon. However, such (hypothetical) uses of DSA say nothing about the intrinsic morality of altering genetic sequences.

Assuming genetic sequence alteration *per se* to be ethically acceptable, it follows that the genetic sequence alteration inherent in gene therapy must also be considered ethically acceptable.

7.7 Animal Experimentation

The development of human germline gene therapy would rely heavily upon the use of laboratory animals. In particular, transgenesis would need to be performed using experimental animals. This would be necessary in order to test and develop delivery systems and transgenes prior to their use with humans.

I have previously suggested that animal suffering gives utilitarian grounds for objection to transgenesis (Smith 2002b). Specifically, I have proposed that *prohibition* should be considered in cases where either of the following negative consequences are entailed:

A. Significant suffering arising in any animals used in the process of transgenesis.
B. Significant suffering arising in transgenics from the development of a pathological condition engineered into the animals' genetic makeup.

I use the term *'significant suffering'* to exclude suffering likely to occur to an animal in a non-experimental situation. For example, animals living in the wild commonly succumb to predators, an occurrence that often involves a degree of suffering, and all animals –including household pets- are at risk from various unpleasant diseases. If the extent of suffering unavoidably entailed by a particular transgenic approach is clearly less than that likely to occur inevitably in the life of the animal, utilitarians have no clear grounds for objection.

Although I propose that prohibition should be considered for negative consequences A & B (above), this should not be taken to mean transgenic cases entailing A or B ought automatically be prevented. Rather, it is necessary, at least in principle, to weigh costs (significant suffering) against potential benefits (for example, a contribution to the development of a new anticancer drug).

From the perspective of suffering, there are two key morally salient features of genetic modification that can lead to negative mental states, such as pain and fear. These features are (i) invasive procedures to recover and transfer embryos, and (ii) killing of animals involved in or arising from transgenesis. Although killing may in principle be free of suffering, I suggest that this is so difficult to achieve in practice that the safest option is to assume *some* suffering, even under the most humane conditions.

It seems undeniable that the process of transgenesis inevitably entails *some* (significant) suffering. The question now becomes: is this suffering outweighed by good consequences? I suggest that -assuming impeccable welfare provisions- an affirmative answer should be given. The 'good consequences' arising from transgenesis may be summarised under the heading of 'scientific progress'. As discussed previously, the scientific value of transgenesis can be in no doubt. Most forms of utilitarianism view scientific progress (in terms of an increased understanding of nature, and of the possible beneficial uses from such understanding) as morally desirable. Thus, prevention of transgenic research *per se* would only be justifiable on the grounds of major negative consequences. I contend that the inevitable significant suffering entailed by transgenesis is insufficiently large to outweigh the benefits to society arising from the contribution of transgenesis to

scientific progress. The amount of significant suffering implicit in transgenesis cannot be quantified. However, the suffering actually entailed by the invasive procedures and killing used in transgenesis ought to be relatively minimal. Typically, donor and recipient animals are used only once in their lifetimes: this is in marked contrast to the many protracted experiments that 'ordinary' laboratory animals endure. Moreover, the procedures themselves are not of a severe nature: at worst (but under proper welfare conditions), embryo collection or transfer is akin to the sterilisation operations commonly used with household pet animals. Similarly, euthanasia is the most frequent fate of pet animals (in using a comparison with household pets to put the process of transgenesis into some context, I assume that most utilitarians share my non-objection to the careful keeping of pet animals).

The *outcomes* of transgenesis that have relevance here are those that are likely to cause suffering. Taking this category of transgenic outcomes in general, the consequences are of the same type as those for transgenesis *per se:* scientific progress is the benefit, and suffering is the cost. However, the degree of suffering implicit in this category of outcomes is greater than is the case for the process of transgenesis. On *prima facie* grounds, I contend that transgenic outcomes that cause significant suffering are contenders for prohibition. I suggest that utilitarians take a 'default' position in which experimentation entailing such negative transgenic outcomes is deemed unacceptable, unless (on a case-by-case basis) a watertight argument has been made to the effect that suffering is clearly outweighed by good consequences. For example, the generation of transgenics that develop an analogue of a painful human cancer ought to be permissible only if the experimenters could clearly demonstrate a major tangible, high probability payoff in terms of a specific advance in cancer treatment. The difficulties in practice of convincingly demonstrating such benefits should not be underestimated: the majority of research using transgenic disease models is *not* expected to yield discernible immediate medical benefits. Moreover, there are many forms of transgenic experimentation for which a cost-benefit justification is *impossible:* for example, pain research may well fall into this category.

By contrast, transgenic research designed to support human germline gene therapy is, I suggest, clearly justifiable on a cost-benefit basis. Protracted or acute significant suffering should certainly not be a key feature of such transgenesis. Moreover,

directed research aimed at developing human germline gene therapy methods would have clear potential utility: as discussed *ad infinitum* in this thesis, GM technology looks set to produce, sooner or later, a revolution in medicine. Thus, the payoffs from transgenesis employed in the pursuit of human germline gene therapy are very high: suffering would be greatly alleviated if effective and safe human germline gene therapy were to become widely available. I conclude that, assuming scrupulous welfare arrangements for the animals involved, the animal transgenesis necessary for the development of human germline gene therapy is ethically justified on utilitarian grounds.

7.8 Future Generations

Future generations of people are morally significant (Ehrlich 2003; Glover 1977; Singer 1993; Van Niekerk 2002). The issue of having permanent effects on future generations renders germline gene therapy especially contentious from an ethical perspective. Given its potential magnitude of effect, utilitarians and others may be tempted to rule germline gene therapy *ultra vires,* through fear that it might cause unalterable and undesirable changes to humanity *per se.* However, there are good reasons to believe that such an absolutist response is one of excessive caution.

Firstly, germline gene therapy should not be singled-out as the only factor able to alter the genetic constitution of future generations. As mentioned previously, medical X-rays on occasion undoubtedly induce mutagenic changes in patients' germline DNA. Environmental factors, ancient and contemporary, damage germline DNA. Moreover, germline mutations are a frequent and inevitable occurrence *in vivo*, regardless of environmental factors. Thus, altering the human germline would hardly represent a new, unparalleled occurrence.

Secondly, the fear of inducing unalterable changes is valid insofar as deleterious (accidental) changes are concerned. However, medically sought germline alterations would be aimed towards beneficial effects – for example, the repair of an oncogene, or the knockout of a dominant disease gene, or the addition of a missing gene

function. From an ethical perspective, such benefits ought to be given weight. Thus, for any proposed germline gene therapy, if the benefit (e.g. a large decrease in cancer risk through the repair of an oncogene) were to outweigh the risks (e.g. insertional mutagenesis), then it would be difficult to ethically defend proscription of the therapy concerned. Moreover, the issue of affecting future generations would in such cases weigh *in favour* of germline gene therapy, since many people (i.e. the descendants of the 'treated' individual) would stand to benefit from the therapy.

Finally, while it is undoubtedly true that mistakes will be made in human germline gene therapy (since mistakes and accidents are an unavoidable feature of all medical procedures, especially new techniques), the same technologies that were used to introduce germline changes could presumably be used to repair accidental genetic damage. In this thesis I have argued that gene targeting would be highly desirable, if not essential, for human germline gene therapy. Assuming human germline gene therapy to be permissible only if gene targeting is available, then a 'faulty' transgene should be able to be repaired or removed by gene targeting, as should endogenous sequences damaged by transgene insertion. Thus, there are strong grounds for delaying human germline gene therapy attempts until the necessary improvements in gene targeting technology have been achieved. However, if and when gene targeting becomes routinely achievable in the human germline, then accidents arising with human germline gene therapy would no longer represent a threat to future generations.

7.9 Gene therapy vs. Genetic Enhancement

If germline gene therapy is in principle ethically acceptable when the technology is perfected, then the question becomes: Which disorders would be serious enough to warrant germline modification? It must fall to ethics committees to decide if a particular disorder is serious enough to warrant germline gene therapy. From a consequentialist perspective, such decisions should in principle be simple: the risks and benefits must be carefully weighed, and the interests of all persons involved must be considered. Of course, many people (in future generations) stand to be affected by

a proposed germline modification, and thus the interests of these future people must weigh heavily in the deliberations of the ethics committee. However, given good scientific knowledge, the actual decision will often be straightforward. To take a hypothetical example: suppose a germline GM process for inactivating a dominant cancer-inducing gene has been shown to be effective in more than 99% animals so treated; suppose also that the risk of a damaging mutational side-effect has been shown to be less that 0.01%; given these scientific facts, the ethically correct course of action would be to permit that particular germline GM process to be carried out on humans. (On the other hand, substantially higher risk / lower success values would of course sway the ethics committee towards the opposite conclusion.) Additionally, the potential for conventional treatment ought to be factored-in to the decision process. In the above example, if the unwanted gene caused a highly invasive, inoperable cancer, this would weigh in more favour of germline gene therapy, compared with the case of a gene causing a slow growing, easily operable cancer.

What of minor disorders, such as colour-blindness or polydactyly? The potential disruption to the parents (due to the associated medical procedures) might well dissuade potential parents from considering germline gene therapy for relatively trivial disorders. In cases where parents nevertheless wished to proceed with germline gene therapy, once again it would be the task of the ethics committee to reach a decision. Again, the previously mentioned principles would apply, with the *minor* nature of the disorder providing *less* weight in favour of approval. And the scope for conventional treatment would be important: for example polydactyly is treatable by simple surgery, whereas no treatment exists for colour-blindness; a lack of conventional treatment would weigh in favour of germline gene therapy. Thus, even for minor conditions, it would in principle be ethically acceptable to perform germline gene therapy, given a high efficacy of treatment and an extremely low risk of deleterious effects. Nevertheless, it is worth re-emphasising that present GM technology is certainly not sufficiently developed to deliver anything approaching the necessary levels of effectiveness and safety.

The ethical problems become greatest in the context of germline genetic enhancement. In contrast to germline gene *therapy,* in which the goal is to avoid or mitigate an otherwise inevitable disorder, *enhancement* implies the manipulation of

general phenotypic traits (where such traits are under genetic control, and hence potentially amenable to GM) to produce 'improved' individuals. Such phenotypic traits might include natural life span, height, disease resistance, IQ, body-shape, size of facial features, etc.

In the future, given safe and effective germline GM technology, it appears inevitable that many prospective parents will wish to employ (and be willing to pay for) germline genetic enhancements for their unborn offspring. Many commentators, including the present author, view the development of a 'genetic elite' - and its corollary, a 'genetic underclass' - as being an ethically repugnant form of eugenics. However, this position ought to be tempered by three considerations.

Firstly, there is a danger that, in attempting to avoid the above-mentioned eugenic scenario, legislators prevent *all* forms of human germline manipulation. Implicit in such notions of prohibition is the view that therapeutic germline GM represents the 'thin end of a wedge' leading inexorably to the reprehensible eugenic use of GM technology described above. As discussed previously, medically useful forms of germline GM may well become available as gene delivery technology improves. It would be highly unfortunate if legislators fail to distinguish between potentially beneficial therapies and undesirable uses of germline GM. It is axiomatic that medico-scientific progress *per se* would be impossible if prohibition were to be applied to technologies simply on the grounds that such technologies *could* be misused.

Secondly, as previously outlined, it should be recognised that no clear boundary necessarily exists between therapeutic GM and enhancement GM. For example, germline GM used against Alzheimer's disease might also be effective in enhancing memory *per se,* in people not predisposed to the disorder. Similarly, germline GM used against a particular inherited cancer might also provide a general resistance against cancer in non-susceptible people. Another example would be germline GM to treat musculo-skeletal disorders, where similar GM applied non-clinically might enhance body-shape or even athletic potential. Although these examples are necessarily speculative and futuristic, they serve to demonstrate that germline GM cannot be simply divided into therapeutic versus enhancement categories.

The final consideration concerning germline genetic enhancements is that some enhancements may actually be desirable for humanity. It would certainly be erroneous to assume that the human genome *per se* is not potentially amenable to improvement. For example, it is now well accepted that evolutionary pressures have been relatively weak in the context of late-onset (post-reproductive age) disorders such as cancer, the result being that humans possess only a modest degree of cancer resistance. Germline GM technology could in principle be used to add 'anti-cancer' genetic sequences (such as extra checkpoint genes or tumour-suppressor genes) into the human genome. Given that these additional anti-cancer genes would be expected to greatly reduce the risk of cancer, it follows that it would be beneficial – and therefore ethically desirable from the utilitarian viewpoint – to carry out this form of germline enhancement as widely as possible, in order that future generations of people were protected against cancer. Examples of human features the improvement or alteration of which by GM might be beneficial to humankind include memory, mood, natural life span, and behaviours such as aggression (Frankel 2000; Resnik 1999; Resnik and Langer 2001; Stock 1999).

The pro-enhancement viewpoint is highly controversial, but it would be a mistake to dismiss it merely as an extremist view, considering the fact that several well-reputed scientists have recently advocated germline genetic enhancement. To take one specific example, James Watson, the biologist who won a Nobel Prize for his role in unlocking the structure of DNA, has recently advocated the use of gene therapy to increase intelligence in persons who would otherwise be born with a low IQ. Of course, support by numbers or support from expert authority does not amount to an ethical case in support of human germline gene therapy. However, I suggest that the pro-enhancement views of those such as Watson are in principle justified on the above-mentioned utilitarian grounds.

7.10 Eugenics

The introduction of genetic enhancements into the human germline would indisputably constitute a form of eugenics, where the term 'eugenics' refers to the improvement of the human gene pool. This is a problem for discussions on human germline gene therapy, because the term 'eugenics' has come to be associated with highly negative, socially coercive attempts by overzealous or totalitarian authorities to control human reproduction. In particular, it is well known that Nazi Germany committed horrific atrocities in the name of eugenics against persons deemed 'genetically inferior'. Consequently, the term 'eugenics' has become so tainted that it may be beyond rehabilitation.

If discussions on the ethics of human germline genetic modification are to progress, then it is necessary to avoid becoming embroiled in rhetorical and pejorative language. Thus, while acknowledging that germline genetic enhancement clearly represents a form of eugenics, I shall attempt to examine human germline genetic enhancement on its own merits and demerits, in a cool and rational manner. Such an approach is central to utilitarian reasoning.

A central issue for human germline genetic enhancement concerns equity. Taking a previous example, it would be advantageous for humanity if an anticancer sequence was engineered into the genome, such that all peoples' cancer risk was significantly reduced. However, suppose that only a subset of parents were able to afford this technology. This subset might plausibly comprise very rich parents in Western nations. The resultant children would form a genetically distinct set of individuals. Moreover, the descendants of such individuals would also be genetically distinct from the rest of the human population. This would be particularly so if the GM (anticancer sequence containing) individuals reproduced only with each other: and it would be rational for such individuals to reproduce in this way, since this would maximise the chances of their offspring also being protected against cancer. (If the 'anticancer' sequence was present (and functional) in hemizygous form, then each offspring from a mating between two GM individuals would have a 75% chance of inheriting the sequence, compared to a 50% chance from a mating between a GM individual and a

non-GM individual. If the 'anticancer' sequence needed to be present in homozygous form, then only GM-GM matings would produce offspring with cancer resistance.) It is immediately apparent that this would effectively split the human species apart, on a most fundamental - and indeed heritable - basis. Utilitarianism does not encompass equality as a good in its own right; however, utilitarian reasoning judges unethical inequalities that are likely to increase suffering.

It seems clear that the division of humanity into GM cancer-resistant and non-cancer-resistant individuals would have a negative effect in terms of the overall balance of happiness/misery in the World. Although the GM contingent would be expected to be happier, through not suffering from cancer, this addition to the net happiness would almost certainly be outweighed by several negative effects. A major effect would be resentment on the part of those who as parents were unable to afford the GM, and also on the part of the non-GM children (and their offspring). It seems unavoidable that such resentment would be magnified whenever non-GM people developed cancer. The likelihood that GM people would refuse to marry non-GM people would further exacerbate the resentment. Such resentment would be bad in itself, because serious resentment is a form of psychological distress, and thus would weigh heavily in the utilitarian calculus. But it would be naive to imagine that the negative effects of such serious resentment would be limited to its direct effects on mental state. Most likely, there would be a risk of civil or even global unrest and strife: it hardly needs saying that a situation of societal breakdown or violence (or at least the risk thereof) would count very strongly against allowing a subset of the population to benefit from GM.

Thus, it seems clear that anticancer GM applied to an elite subset of Homo sapiens would have deleterious consequences. Yet it should be noted that this example of GM lies on the milder, less controversial and divisive end of the human germline genetic enhancement spectrum. Anticancer GM would be of medical benefit (albeit preventive), but it would not impart any further advantages on the GM individuals concerned. Human germline genetic enhancement designed to improve certain non-medical parameters would be even more divisive to humanity. Take for example a GM elite with enhanced mental functioning, such as better memory or higher IQ or freedom from anxiety. Such individuals would be able to secure for themselves and their offspring economic advantages; moreover, they might well consider themselves

as inherently superior to their non-GM counterparts. If I am correct in positing overall negative consequences from the use of anticancer GM to create an elite group, then the case is even stronger against germline genetic enhancement for non-medical features.

To a large extent, the obvious severity of the bad consequences likely to arise from elitist use of human germline genetic enhancement should be expected to function to prevent such disasters from occurring. It is, I hope, not unduly optimistic to expect that societies that obtain the tools for germline genetic enhancement would prohibit its use for non-medical, socially divisive purposes. Certainly, as long as scientists and ethicists are able to examine each proposed germline genetic enhancement with precision, it should not be difficult to predict which enhancements to permit and which to prohibit, when particular individuals or groups (e.g. the wealthy) are involved. Of course, individual countries might be unwilling to conform to international rules, and this would present a problem for the World community.

Thus far in this discussion of human germline genetic enhancement, I have assumed that particular individuals might wish to secure advantages for themselves through GM, and in this context it seems clear that human germline genetic enhancement ought to be very heavily circumscribed. However, there may well be justification in permitting germline genetic enhancement for persons who are in special need of particular enhancements. In other words, it may be justifiable to use human germline genetic enhancement to give some individuals a helping hand (as opposed to an advantage) in life. For example, consider parents of very short stature due to genetic factors. To avoid a similar very low stature in their children, in principle germline genetic enhancement could be offered to such parents. A more controversial example would be parents of genetically conferred low IQ. Of course, in neither case is it easy to draw a line whereby treatment should be offered: exactly what predicted height or IQ in a child ought to be deemed low enough for germline genetic enhancement to be invoked? There are no easy, clear-cut answers to such problems: drawing such lines is inevitably somewhat arbitrary. Ethics committees must do their best in such cases to produce consensus decisions. But the fact that drawing such lines is difficult should not detract from the utilitarian consideration that enhancements designed to ameliorate disadvantages should increase overall happiness.

This sort of case is made more complicated by the fact that the genetic basis of many important features – probably including height and IQ – is of the polygenic variety, and thus it is more difficult to predict the phenotype of future offspring. Moreover, where human features – especially psychological ones – are under polygenic control, it is worth considering that some of the genetic variation may be beneficial. Put simply, the use of GM to alter the genetic constitution of those who manifest extreme phenotypes might have the deleterious side effect of removing intrinsically useful alleles from a genetic lineage. At present, such considerations are unavoidably speculative: our understanding of the molecular genetics of human behaviour is at a rudimentary state of development. We simply do not know whether human germline genetic enhancement to promote, say, less aggressive behaviour might remove genes that promote (say) perseverance. A similar example might be autistic personality features and mathematical ability: by removing genes implicated in the former by human germline genetic enhancement, might genes conferring the latter be diminished? Given the present lack of molecular genetic elucidation of human behaviour and psychology, all that can be done concerning this issue of polygenic traits and human germline genetic enhancement is to recognise it as a potential problem, and one that may or may not prove to be of significance. Fortunately, just as the technologies that would allow human germline genetic enhancement are developing, the molecular genetics of the human condition are beginning to be unravelled. It is probable that, when human germline genetic enhancement becomes a realistic prospect, science will have amassed a thorough understanding of the genes underlying contender phenotypes for enhancement.

This discussion has been considering the applicability of human germline genetic modification to subgroups of the human population. I now turn to the possibility of applying human germline genetic modification to all of humankind. Just as vaccination against serious infectious diseases has been applied to as many children as possible, in the future human germline genetic modification may come to applied globally, to the greatest number of (future) people possible.

Such global possibilities would alter the ethical status of certain genetic enhancements. For example, I previously suggested that anticancer germline genetic

enhancement would be a great disutility if its use was restricted to an elite subset of humans. However, if the same GM process were to be made available to all (or as many as practically possible) people, then the benefits (less cancer-caused suffering and fewer premature deaths) would not be accompanied with the problems associated with GM elitism (resentment, social breakdown and strife). I suggest that such medical enhancements are supported by utilitarianism, since the benefits would be so great.

However, non-medical germline genetic enhancements are more problematic for utilitarians. Whereas there would be obvious benefit in improving the height or IQ of future persons otherwise destined to become very short or very unintelligent adults, it is not obvious that increasing the height or IQ of all persons would lead to an increase in happiness – especially if the relative differentials were maintained. Certainly, a very strong case would need to be established in terms of the potential utility of any such global non-medical application of human germline genetic modification.

Another problem for global human germline genetic enhancement concerns polygenic traits. I have suggested that germline genetic enhancement of unwanted personality or behavioural traits may deplete a particular genetic lineage of desirable genes. This same concern applies with global germline genetic enhancement, but with more force in view of the fact that any loss of desirable genes would involve the entire human gene pool. Again, detailed molecular knowledge of the genes involved in human psychology and behaviour would be required before any such germline genetic enhancement could be ethically countenanced.

If global human germline genetic modification was to become possible, it would require a methodology that would be easy to apply, would not require a complex biomedical infrastructure, and would be inexpensive. As outlined in the section on cost (7.4), this would almost certainly have to involve some form of SMGT. The possibility that SMGT comes to be developed such that it could be easily applied – as has already been claimed with some recent agricultural transgenesis experiments (Lavitrano et al. 2003) – suggests the possibility of some ethically very potentially perplexing situations arising. The central problem is that a cheap, easily applied

human germline gene transfer technique could allow human GM to be carried out in situations beyond the control of democratic government.

One such situation might be human germline genetic enhancement offered by private commercial bodies, in countries with relatively lax laws on biomedical procedures. Just as some people have shown themselves to be willing to select the sex of their children (either by prenatal sexing followed by embryo/foetus discard, or – less reliably – by sperm separation techniques), it seems inevitable that some people would be willing to procure genetic advantages for their offspring, if the technology becomes commercially available. Given the foregoing ethical objections to human subgroups forming genetic elites, it is clear that utilitarians should be opposed to such commercial trends.

Perhaps, more worryingly on account of the potential scale of the problem, would be the situation in which one government took a unilateral decision to apply human germline genetic enhancement to many or all of the country's (future) inhabitants. Some countries have demonstrated a willingness to push ahead technologically, and are unlikely to be constrained by the bioethical considerations and processes that have developed in the Western world. China is of interest here (Dikotter 1998; Doring 2003; Mao 1998). It is well known that the government has been vigorous in applying reproductive control over its subjects. Such control includes the one-child per family policy, laws requiring premarital genetic health checks, and even compulsory sterilisation for some genetic disorders. Moreover, China has embraced GM technology: several GM crops have been created by (and are now grown in) China, and the country's first somatic gene therapy product has recently been approved for general use. One may reasonably speculate that, should SMGT-based human germline GM become available, non-democratic, technologically advanced counties such as China may be the first to apply it – possibly regardless of individual parent's preferences and possibly without thorough bioethical discussion.

To utilitarians, any piece of technology is ethically neutral: ethical considerations apply to questions concerning the uses to which the technology is applied. Accordingly, SMGT-based human germline genetic modification would be ethically neutral; but its inception would raise many bioethical issues. Thus, there is an

interplay been the basic science & technology of human germline GM on the one hand, and bioethics on the other. As humanity is poised to enter a new revolution in genetic science, utilitarianism represents a powerful bioethical tool that should be promoted for use amongst all concerned, from ethics committees to state governments.

Chapter 8: Conclusions: the Prospects for Human Germline GM

Genetic modification technology is steadily becoming more effective. There is now a broad armamentarium of gene transfer methods available to deliver transgenes to a broad range of cell types. Several of these methods have proved effective in generating transgenic mammals including primates, and some of these methods could almost certainly be used with a minimum of adaptation to genetically modify the human germline.

However, the most established germline gene transfer method, pronuclear microinjection, has a number drawbacks, including expense, low overall efficiency, unreliable/unpredictable transgene expression, and a relatively high risk of endogenous genetic damage. Accordingly, it would be difficult in practical terms, and unethical from the perspective of risk to the (unborn) 'patient', to attempt human germline gene therapy using pronuclear microinjection.

Although not in widespread use in animal transgenesis, retroviral transfer is an established method that has the advantage of a relatively high overall efficiency. Moreover, retroviral transfer (with RVVs being physically microinjected into eggs) has been the only method to have produced transgenic primates. However, control over transgene expression is limited, and the risk of genetic damage is higher than with pronuclear microinjection. Thus, as with pronuclear microinjection, bioethical considerations concerning patient safety vs. the probability of an effective result dictate that retroviral transfer at its present level of development is not feasible for use in human germline gene therapy.

An alternative approach is SMGT. In reports of successful SMGT-based animal transgenesis, the approach appears to offer high overall efficiency levels. Moreover, SMGT offers the possibility of a low cost, relatively low-tech means to germline GM; this would in principle make human germline gene therapy widely available. However, SMGT has not yet been established as a reliable form of transgenesis, especially in its (potentially most useful) *in vivo* and AI modes. Furthermore, problems of unreliable/unpredictable transgene expression, and a relatively high risk of endogenous genetic damage, are likely to apply to SMGT much as they apply to pronuclear microinjection.

As science and technology progress, it is to be expected that the foregoing methods of gene transfer will be gradually improved, such that they are more efficient. However, all of these methods are inherently limited in that transgene integration takes place into random sites within the host genome. It is this random mode of integration that underlies the expression problems and genetic damage risks that accompany gene transfer.

While improved transgene design may ameliorate some of these problems, the only known way to avoid these problems entirely is to target transgenes to chosen genomic loci. Indeed, gene targeting has the potential not only to avoid problems of expression and damage problems, but also to alter the host genome such that endogenous genes can be removed, replaced or repaired. Clearly, gene targeting is the ultimate tool for human germline gene therapy.

Gene targeting is routinely achieved in animal transgenesis. However, the standard approach involves the use of totipotent ESCs, which to date have only been established for mice. It is not known whether totipotent ESCs could be developed for humans: certainly, human ESCs would be a major breakthrough for human germline gene therapy because gene targeting in the human germline would become possible. Regrettably, however, bioethical constraints effectively exclude the research necessary for the establishment of human ESCs. This is not (from a utilitarian perspective) due to fact that many human embryos would have to be experimented upon; rather, the problem is that the only way (as far as can be foreseen) to demonstrate totipotency would be to use human ESCs to produce human chimeras that develop successfully into an adult. Thus, ESC-based gene targeting does not look like a practical proposition for human germline gene therapy.

In recent years, NT-based transgenesis has been developed. This approach has permitted gene targeting in a variety of (non-murine) animals. It is likely that, given suitable empirical modifications, NT could be used to achieve gene-targeted human germline gene therapy. If this was ever to happen, it would also be necessary for the process to be made more efficient, since in its present form, NT requires a supply of eggs that would render the approach impractical for human applications. More

fundamentally, the 'founder' animals produced via NT have very high rates of debility and developmental abnormality, probably due to epigenetic damage. Until such time as the source of these health problems comes to be eliminated, it would be unethical to use NT for human germline gene therapy.

Research into the parameters controlling HR, the underlying process of gene targeting, continues apace. From such research it is to be hoped that the efficiency of gene targeting can be substantially improved. If gene targeting could become the norm, and random integration a minority event, then it might be possible to achieve gene targeting without the need for *in vitro* selection. If so, gene targeting in human germline gene therapy could be achieved without the need for ESCs or NT.

There is a need for more systematic research into transgene design, such that targeting transgenes can be designed that will give optimum targeting efficiencies. However, it is unlikely that adjusting transgene sequence alone will prove sufficient to improve the rates of targeted integration to levels that would allow selection-free gene targeting. A second necessary avenue for exploration concerns the recombinase enzymes that drive HR. Again, research is gathering pace into this presently little understood area of molecular biology. Speculatively, it may be possible to co-transferred potent recombinase enzymes or enzyme complexes (natural or synthetic) alongside/attached to transgene molecules during gene transfer, such that HR is stimulated in the host cell, resulting in high efficiency gene targeting.

To achieve this, not only would there need to be a much-enhanced understanding of the processes of HR, it would also be necessary to design appropriate transgene delivery systems for gene targeting. While the most conceptually straightforward way to co-introduce recombinase molecules would be pronuclear microinjection, this would still encumber human germline gene therapy with a technically difficult basic procedure. It would be desirable if instead of pronuclear microinjection, *en masse* delivery systems could be improved and adapted to co-deliver recombinase enzymes. In this regard, liposome-mediated gene transfer represents a promising area. Intensive efforts are underway to improve liposomes (primarily for somatic gene therapy applications) in terms of cell specificity, cell-uptake efficiency, and nuclear localisation ability. If such efforts are fruitful, it may be possible that in the future

such liposomes could be used to deliver transgenes plus recombinase molecules to gametes, possibly *in vivo*. In this regard, transgene delivery systems are moving into the rapidly developing sphere of nanotechnology.

This thesis has explored the ethics of human germline gene therapy from a utilitarian perspective. From this perspective, several conclusions follow. These conclusions form the remainder of this chapter.

In technical terms, it is clear that – unless an unpredictable 'quantum leap' breakthrough occurs – it will take several years of development before germline GM methods become sufficiently reliable, safe and cost-effective to allow human germline gene therapy to be attempted. Practical problems (such as the need for many human eggs from a parent) aside, the relatively low effectiveness of present germline GM technology would not be enough to ethically justify exposing a 'patient' to the associated genetic damage risks. This proscriptive position is reinforced by the impossibility of obtaining consent from the 'patient' in human germline gene therapy. Moreover, genetic pre-screening is presently a safer proposition that human germline gene therapy, and most of the conditions potentially amenable to human germline gene therapy are also inherently avoidable via pre-screening.

Thus, human germline gene therapy is prohibited at present on straightforward ethical grounds. However, if and when gene transfer technology improves such that human germline gene therapy becomes efficient, effective and safe, utilitarians have no safety grounds for maintaining a prohibitive stance. Indeed, it is probably true that human germline gene therapy accords better with people's natural inclinations than does pre-screening, since the latter involves elimination of life whereas the former is life-enhancing – a side effect that would lend some support to human germline gene therapy over pre-screening, where the two options were otherwise on a par.

The public's response to human germline gene therapy represents opinion that should be considered by policy makers, but the public's response is not an ethical entity of itself. Utilitarian deliberations need to consider the public's view of human germline gene therapy as a side-effect. Although there may be a strong reaction against human germline gene therapy, this is anticipated to be culturally sensitive rather than

reflecting any deep-seated intuitive emotions. It seems likely that, if the public can be shown the potential medical value of (safe & effective) human germline gene therapy, support will be given.

The cost of human germline gene therapy and associated limitations of access to human germline gene therapy are of ethical concern. If human germline gene therapy were to be available only to an elite subset of humans, this would quite probably lead to social division. This would be especially so in the case of genetic enhancement, as opposed to gene therapy. Accordingly, utilitarianism would not support 'elitist' human germline gene therapy. However, if the sort of improvements in gene transfer technology outlined previously are ever realised, such that human germline gene therapy became cheap and easy to apply, then ethical concerns over the costs of human germline gene therapy and access to it would largely disappear.

The issue of future generations of people is important in utilitarian discussions of human germline gene therapy, since the effects of germline GM are not limited to individual patients but apply also to subsequent generations – and hence ultimately to the entire human gene pool. Thus, any mistake made with human germline gene therapy may threaten many (future) people, or even humanity itself. However, mistakes are likely to be repairable by the high tech procedures that would be used for human germline gene therapy. Moreover, human germline gene therapy would be directed at improving the human germline. Therefore, accidents aside, the fact that future generations stand to be affected by human germline gene therapy represents a beneficial consequence.

Genetic enhancement, as opposed to gene therapy, is a difficult issue in ethical considerations of human germline gene therapy. This is so because there is no clear dividing line between purely therapeutic applications of human germline genetic modification, and alterations designed to improve non-medical phenotypic features. Utilitarianism is supportive of the former, but somewhat prohibitive of the latter, since therapeutic GM should increase overall happiness (less disease) while enhancement GM may lead to psychological distress and even social breakdown. However, it is important to realise that some people may legitimately benefit from genetic enhancement (e.g. those destined to have a low IQ would benefit from genetic

correction to produce a normal IQ), where the same GM might otherwise be employed to give an unfair advantage to those who have no legitimate requirement for the enhancement (e.g. people of normal intelligence).

Finally, the issue of eugenics is central to ethical consideration of human germline gene therapy. However, the term 'eugenics' is rather pejorative, and should not be permitted to function as a rhetorical device to prevent rational debate. One valid utilitarian argument that needs to be made is that provided desirable genes are not inadvertently eliminated, the use of human germline gene therapy to improve the human gene pool (by reducing the frequency of deleterious alleles) should be recognised as a worthy goal.

Nevertheless, several risks remain. Use of human germline genetic modification by one subset of people would threaten to establish a genetic elite – a situation of disutility considering the probable psychological and societal effects that would result. The situation could be even worse if an entire country was to unilaterally apply human germline genetic modification, especially if this was for enhancement purposes.

Clearly, these ethical problems, and their solutions, are located in the domain of social policy and politics. Moreover, these problems are not of immediate concern, since the requisite GM technology has not yet been developed sufficiently to permit human germline genetic modification. However, despite the relative infancy of GM technology, it is salutary to remember that the germlines of many mammals including primates have already been successfully genetically altered. Considering the accelerating pace of genetic science, it seems inevitable that technological improvements will over the forthcoming years place such ethical problems firmly into the territory of applied ethics.

Appendix I

The pages 303-305, containing the full text of the published article cited below, have been removed from the e-thesis due to copyright restrictions:

Smith, K.R. (2003). The ethics of anomalous, unconventional therapies: a utilitarian response. In *The Scientific Review of Alternative Medicine*, 7(1), pp.26-28.

Appendix II

Population Ethics: A Hedonistic Response to Parfitt's Repugnant Conclusion

1. Introduction

In his seminal work *Reasons and Persons,* Derek Parfitt claims that 'total view' consequentialism (TVC) generates the *Repugnant Conclusion*:

> RC: for any large population of people, all with lives well worth living, there will be some much larger population whose existence would be a better alternative even though its members all have lives that are only barely worth living (Parfitt 1984).

In the descriptive terms used by Parfitt, the large population of people all with lives well worth living, experience "Mozart", whereas the much larger population all with lives only barely worth living, experience "musak".

As Parfitt's negative term 'repugnant' suggests, it is difficult to accept RC as a good consequence. However, a rejection of RC seems to entail the rejection of TVC. I shall refer to this *prima facie* paradox or tension as RC/TVC. Parfitt considers and rejects various attempts to resolve RC/TVC.

2. Argument Framework and Premises

My arguments are expressed within a framework of hedonistic (mental-state) utilitarianism. (This form of consequentialism is employed for the sake of simplicity; alternative forms, such as Desire Fulfilment or Act Utilitarianism could also be used. However, the reasoning entailed would be highly complex.) The force of this response from hedonism rests upon certain premises, as follows:

> P1 Pleasure exits in different forms. (This view is also held by Parfitt. It is opposed to classical views of pleasure (such as that of Bentham), but

should not be confused with Mill's notions of 'higher'and 'lower' pleasures[1]

P2 Generation of pleasure depends ultimately upon energy and matter in the physical sense, and;

P3 Energy and matter (call them 'Resources') are in limited supply within the Universe. Furthermore, his is a *deeply true* fact; it would apply to any conceivable universe. (An infinite universe *might* change P3 – but even within such a universe it might not be practical to actually access Resources in a limitless way.)

3. Hierarchy of Pleasure

For the sake of simplicity, arguing from P1 I shall deal with only two different forms of pleasure. One is very low level, call it 'Basal'. This is akin to Parfitt's 'Musak': Basal refers to the state of mind prevailing when only very basic needs are met.

The other form of pleasure is high level, call it 'Ultra'. This is akin to Parfitt's 'Mozart'. However, note that Basal and Ultra refer specifically and absolutely to mental states; Parfitt's Musak and Mozart imply environmental experiences, leaving the corresponding mental states somewhat unclear.

The possible existence of Basal and Ultra appears incontrovertible. (Of course, there may exist a gradient comprising more than two forms of pleasure; knowledge here awaits scientific (e.g. neurobiological) elucidation. This in no way alters the basic tenor of the argument from hedonism.) Extending from this, two plausible claims may be made:

C1 A person cannot experience Ultra unless Basal is already present.

C2 Basal and Ultra each have intrinsic value.

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[1] See Scarre (1996) for an overview of the views of Bentham and Mill on pleasure.

From C2, it may be asked: Are the intrinsic values Basal and Ultra *directly additive*? On *prima facie* grounds at least, this does not appear likely. If P1 ("pleasure exists in different forms") is correct, because Basal and Ultra are different forms of pleasurable mental states, then it becomes an open question as to whether a Basal value and an Ultra value can be added together.

In general, two different entities cannot be added together. A simple but pertinent analogy is that of apples and oranges: 3 apples + 2 oranges = [3 apples and 2 oranges]; it would be absurd to claim that 3 apples + 2 oranges = 5 of any entity. (Of course, it can be said that 3 apples + 2 oranges = 5 fruits, but this is merely a semantic fix.)

It may remain mathematically possible to add values for separate entities such as Basal and Ultra. However, the onus of proof must rest with those wishing to reject the common-sense notion that Basal and Ultra are not additive. Thus, I make the following claim:

C3 Intrinsic values of Basal and Ultra are not directly additive.

This claim is central to the argument from hedonism.

4. Compensation?

Can extra people compensate for a reduction in Ultra? According to C2, Basal and Ultra each have value. Suppose, for simplicity, that there is a one-to-one relationship between the two mental states. In numerical terms, assume that one unit of Basal is equivalent to one unit of Ultra. It is important to note that this equivalence derives from *the quantity of Resources required to produce pleasure.* Thus, although the 'Units' are measures of pleasure, this does *not* imply that the two mental states are additive.

Now compare two very small possible worlds:

W1 This world has a population of two people. Both experience Ultra. From C1 & C2, Table 1 provides a summary of the relative amounts of pleasure involved:

Table 1	Units of Pleasure in W1	
Basal	1	1
Ultra	1	1
Person	I	II

W2 This world has a population of four people. All experience Basal, but none experience Ultra. Table 2 summarises this:

Table 2	Units of Pleasure in W2			
Basal	1	1	1	1
Ultra	0	0	0	0
Person	III	IV	V	VI

Which is the best world? According to RC, neither world is better than the other. I suggest that this view is mistaken. Both worlds contain 4 Units of pleasure. However, only W1 contains the higher type of pleasure. Thus, at least on *prima facie* grounds, we ought to prefer W1 to W2.

So, in this simple comparison between W1 and W2, it would appear that extra people are inadequate compensation for the loss of Ultra.

5. Consolidation

Suppose we have to decide between two alternatives, as outlined below.

W1 has the opportunity for increased pleasure, due to the discovery of additional Resources. Suppose that the decision is constrained between (a) not utilising the new Resources and therefore keeping W1 unchanged, or (b) utilising the new Resources, such that W1 changes to $W1^{+}$, as shown in Table 3:

Table 3	Units of Pleasure in $W1^{+}$					
Basal	1	1	1	1	1	1
Ultra	1	1	0	0	0	0
Person	I	II	VII	VIII	IX	X

From Table 3, there seems to be no doubt that $W1^{+}$ is better than W1.

However, if the decision were not constrained as above, rather than utilising the new resources to increase population size, it would have been better to apply a *Principle of Consolidation.* This principle is based on my previous claim that, despite the total Units of pleasure remaining numerically unaltered, extra people are *prima facie* inadequate compensation where a loss of Ultra is entailed. Consolidation operates as follows:

Consolidation: Resources should be allocated such that, for a given total number of Units of pleasure, the maximum level of Ultra should be attained.

If Consolidation is applied to W1 facing a decision on how to utilise new resources, the best possible world ($W1^{*}$) would result:

Table 4	Units of Pleasure in $W1^{*}$			
Basal	1	1	1	1
Ultra	1	1	1	1
Person	I	II	VII	VIII

So, Consolidation should, wherever possible, be applied to the question of utilisation of Resources to generate pleasure. Consolidation is therefore an important principle when considering "more people *vs* a higher quantity of pleasure" dilemmas. Consolidation forms a central part of the argument from hedonism.

Now consider two alternative populations, *A* and *Z*. (The designations *A* and *Z* are taken from Parfitt.) Population *A* comprises individuals all with lives well worth living. Population *Z* is a larger population, the members of which have lives that are only barely worth living. These two alternatives may be expressed thus:

Table 5	Population *A*	Population *Z*
Persons (numbers x 10^6)	100	1,000,000
Basal (Units of Pleasure)	100	1,000,000
Ultra (Units of Pleasure)	100	0

Which is best, *A* or *Z*? Of course, the Repugnant Conclusion suggests that *Z* wins. As Parfitt's negative term 'repugnant' suggests, it is difficult to accept this. We need to consider: Is it likely that, through time, Consolidation could be applied to *Z*? In other words, could population numbers be reduced (by birth control) and Resources reallocated to generate ultra? If so, Consolidation would convert Population *Z* into a population comprising fewer persons, each with a balanced allocation of Basal and Ultra:

Table 6	Population *Z**
Persons (numbers x 10^6)	500,000
Basal (Units of Pleasure)	500,000
Ultra (Units of Pleasure)	500,000

Population *Z** is clearly better than either *A* or *Z*. Therefore, going back to the *A vs Z* judgement, we should prefer *Z* if Consolidation is possible.

However, suppose that it becomes absolutely impossible, in the near or far future, to apply Consolidation (due perhaps to the rise to total domination of a government of extremists?) within Populations *A* or *Z*. In this case, I believe that *A* must be preferable to *Z*, for the following reasons:

> From C3 (intrinsic values of Basal and Ultra are not directly additive), it would be *invalid* to add together Basal and Ultra pleasure values within *A* ("100 + 100 = 200") to compare it with the quantity of pleasure in *Z* (1,000,000). Therefore, no valid conclusions can be drawn from such a comparison. This leaves only the difference in *types* of pleasure as a valid basis for comparison: since only *A* contains the higher type of pleasure, I conclude that *A* is preferable to *Z*.

Would this conclusion still hold if the differences between *A* and *Z* were even sharper? For example:

Table 7	Population $A^{\otimes}$	Population $Z^{\otimes}$
Persons (numbers x 10^6)	10	1,000,000,000,000
Basal (Units of Pleasure)	10	1,000,000,000,000
Ultra (Units of Pleasure)	10	0

Again, the argument from C3 gives the same answer: $A^{\otimes}$ is preferable to $Z^{\otimes}$.

The problem here is that this argument seems to be generating an *absurd* conclusion, where phenomenally huge populations experiencing only Basal are deemed worse than much smaller populations experiencing Ultra. So, RC has been avoided, but the alternative "absurd" conclusion is similarly difficult to accept.

But does this apparent *reductio ad absurdum* actually hold? It is highly difficult, if not impossible, to envisage a realistic scenario in which Consolidation is absolutely unattainable, even in the distant future. (Even a very small degree of Consolidation in $Z^{\otimes}$ (for example, a population reduction of 0.000000001%) would render $Z^{\otimes}$ better

than $A^{\otimes}$.) Because a situation of permanent non-Consolidation seems highly implausible, I conclude that no absurd conclusions are implied in the argument from hedonism.

6. Conclusion

Premised upon (a) the existence of a Hierarchy of Pleasure and (b) a Principle of Consolidation, the argument from hedonism proposed in this appendix offers a possible way of avoiding Parfitt's Repugnant Conclusion. In so doing, this argument may save Total View Consequentialism from the rejection implied by the Repugnant Conclusion, and thus supports the utilitarian tradition inherent in population ethics.

Appendix III

The pages 316-319, containing the full text of the article cited below, have been removed from the e-thesis due to copyright restrictions:

Smith, K. (2002). Problems of affluence in morality. In *Philosophy Now*, October/November, pp.28-31.

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